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Effectiveness of nanobioinsecticide against the mushroom Sciarid, *Lycoriella auripila* (Diptera: Sciaridae)

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Plant oils, because of their diverse bioactive properties, are considered suitable candidates for formulating biopesticides. This study aimed to investigate the insecticidal potential of a nanoformulation containing: cinnamon, coconut, neem, eucalyptus and citronella oils, against *Lycoriella auripila* flies in an edible mushroom cultivation medium. The efficacy of these formulations was compared to that of conventional synthetic insecticides, specifically thiamethoxam (25% Water Dispersible Granule, WG) and imidacloprid (35% Suspension Concentrate, SC). The prepared nanoemulsion was characterized using various techniques including gas chromatography, transmission electron microscopy, scanning electron microscopy, dynamic light scattering and gas chromatography-mass spectroscopy. In a contact bioassay conducted on adult insects, the LC_{50} value of the nanobioinsecticide was recorded as 765.97 mg a.i. l^{-1}. However, the LC_{50} values of the chemical insecticides showed higher toxicity, with imidacloprid at 116.41 mg a.i. l^{-1} and thiamethoxam at 201.08 mg a.i. l^{-1} proving more effective than the nanobioinsecticide against adult sciarids. By contrast, when tested against larvae, the high-concentration nanobioinsecticide achieved 92% mortality at 7 days post-treatment, whereas thiamethoxam and imidacloprid demonstrated 52% and 71% efficacy, respectively. Overall,

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these findings suggest that the developed nanobioinsecticide shows promising potential for managing mushroom sciarids and could be effectively integrated into an integrated pest management programme within edible mushroom production facilities.

1. Introduction

Mushroom sciarid flies (Diptera: Sciaridae), commonly known as dark-winged fungus gnats or compost flies, are commonly found in moist environments. They are considered major pests in mushroom farms, household plant pots and greenhouse crops. Among the various species, those belonging to the genera *Bradysia* and *Lycoriella* are particularly damaging. *Lycoriella* species, in particular, are predominantly found in commercial mushroom cultivation systems, where they can cause extensive economic losses [1]. In Iran, chemical control is the most conventional method for managing the sciarid flies. Mushroom production facilities are constantly threatened by these pests. Unchecked populations can lead to significant crop loss owing to both direct damages caused by larval feeding and indirect damage through pathogen transmission by adults [2,3]. In the UK, *Lycoriella auripila* (Winnertz) has been reported to exhibit high resistance to organophosphorus insecticides [4].

To reduce pesticide application, researchers have recently turned to biopesticides [5]. Biopesticides are compounds used to manage agricultural pests [6,7]. It appears that plant components may replace the chemical pesticides in the future. Compared to chemical pesticides, biopesticides are safer because of their biodegradable nature, lack of persistence, rapid disintegration and minimal negative effects on groundwater, along with their agroprotective qualities [8,9]. Plant-based pesticides can be used separately or in combination with other compounds to target a range of insect pests affecting crops [10].

With the introduction of emerging technologies, such as agricultural nanotechnology, it is anticipated a significant milestone in this field [11]. Nanotechnology plays a crucial role in developing environmentally safe, target-oriented and controlled-release pesticides [12]. Nanoformulation enhances solubility, dispersion, and bioavailability, preventing premature degradation and enabling tailored release of plant-based pesticidal components [13,14].

One effective way to reduce the use of chemical pesticides is by using organic insecticides. Repellents have substantial untapped potential when integrated into pest control systems. Certain plants possess a natural ability to repel insects, often outperforming synthetic materials [15]. By using formulations that incorporate repellents in nanoparticles, it is possible to decrease dosage requirements and prolong the release time of active compounds. This approach not only reduces toxicity but also minimizes the number of treatments required [16,17].

In recent years, there has been a significant drive to develop essential oils of botanical origin as potential agents for pest management techniques [18]. Neem, also known as *Azadirachta indicia*, is frequently used as a natural substitute for N,N-diethyl-meta-toluamide (DEET) in India and several neighbouring countries. It has been tested for insect repellent against various arthropods with varying degrees of success [19,20]. Neem components exhibit a variety of effects on insects, including flies, houseflies and cockroaches [21,22]. Neem oil, known for its natural insecticidal compounds such as azadirachtin [23], has been combined with other bioactive essential oils and formulated into a stable nanoemulsion. Citronella, known for its ability to produce long-lasting repellents, uses microencapsulation technologies to improve the efficacy of natural repellents [24]. Citronella grass (*Cymbopogon nardus* L.) emits a characteristic scent and serves as an effective pesticide [22,24]. One of the active ingredients in eucalyptus, 1,8-cineole (eucalyptol), has been shown to be a potent oviposition repellent and a moderate repellent for feeding flies [25]. The aroma and flavour of cinnamon are derived from the essential oil and its main component, cinnamaldehyde, as well as several other compounds, including eugenol [26,27]. Cinnamon essential oil contains phenylpropanoids, monoterpenoids, sesquiterpenoids and oxygenated sesquiterpenoids [28]. Additionally, coconut oil contains lauric acid, myristic acid, caprylic acid, palmitic acid and capric acid, all of which possess antimicrobial properties [29]. These widely cultivated plants are simple to grow and serve as effective pesticides and insect repellents.

Through the nanoformulation process, the active ingredients are protected from rapid degradation and volatilization, allowing for sustained release and prolonged action against the target pest. This study aimed to investigate the potential of a nanobioinsecticide against *L. auripila* flies (sciarid fly), a major pest affecting mushroom medium. The insecticidal effects of mixed botanical ingredients were

evaluated across different developmental stages as well as on adult flies. The findings contribute to the advancement of biopesticide research using combined natural compounds and highlight the role of nanoformulations in promoting sustainable agricultural practices.

2. Material and methods

2.1. Materials and equipment

Coconut oil, eucalyptus and cinnamon essential oils were obtained from Jahad Daneshgahi Alborz (Iran). Citronellal essential oil, Tween 80 and Span 60 were obtained from Merck Schuchardt OHG (Germany). Neem oil was supplied by Naissance UK. Chemical reagents such as sodium chloride, sulfuric acid, methanol, acetone, hexane, ethanol (96%) and acetone were provided by Sigma-Aldrich. Furthermore, an aliquot of nanoemulsion was used to investigate morphology and particle size by scanning electron microscopy (SEM) using (MIRA2, TESCAN, Czech Republic) and transmission electron microscopy (TEM; Tem Philips em208s, 100 kv). Dynamic light scattering (DLS; Particle Metrix, Stabilizer 200, Germany) was used to determine the average particle diameters and the relative distribution of particle size. In this study, two neonicotinoidal insecticides were used as reference chemical controls: thiamethoxam 25% WG) (Actara®; Syngenta, Iran) and imidacloprid 35% SC) (Bahavar Shimi Company, Iran).

2.2. Preparation of essential oil nanobioinsecticide

The nanoemulsion was prepared using a two-step process to emulsify oil in water. First, 500 µl of Tween 80 was mixed with 200 µl of acetone and mixed at room temperature (RT) for 10 min under gentle mixing to form the surfactant solution. Next, 1 g of Span 60 (at a final concentration of 1%) was dissolved in 100 ml of deionized water and stirred for 5 h at 40–45 °C and 1400 rpm to ensure complete dissolution. Subsequently, 923 µl of Tween 80 was gradually added to 77 µl of the 1% Span 60 solution under continuous stirring at 1400 rpm for 1 h to obtain a homogeneous surfactant mixture. The plant-derived oils and essential oils, including neem oil, coconut oil, eucalyptus oil, cinnamon oil and citronella oil, were then added dropwise to the surfactant phase in a volumetric ratio of 4 : 9 : 2 : 1 : 1, respectively. This addition was carried out in the dark over a 30 min period, while maintaining vigorous stirring, without altering the temperature. Throughout the process, the pH of the emulsion was monitored after each addition and remained within the range of 5.03–7.20, indicating a formulation suitable for biological applications.

2.3. Fatty acid methyl ester

2.3.1. Extraction and preparation of neem and coconut oil samples

To prepare a 2% sulfuric acid solution in methanol, we combined 100 µl of sulfuric acid with 4900 µl of methanol. For the 0.9% sodium chloride solution, we dissolved 0.09 g of sodium chloride in 10 ml of solvent [30]. Neem and coconut oil (20 µl) were added to a 2 ml vial, followed by the addition of 300 µl of the 2% sulfuric acid solution in methanol. The vials were carefully covered with parafilm to prevent methanol evaporation and then vortexed for 20 s. Next, they were placed in a bain-marie shaker at 80°C and 200 rpm for 2 h. Afterwards, the vials were cooled at RT for 5 min. In the next step, 300 µl of the 0.9% sodium chloride solution and 300 µl of hexane were added and vortexed for 20 s, followed by centrifugation for 5 min at 20°C and 3000*g* until a biphasic mixture formed. A 1 µl sample was taken from the upper phase. At this stage, the methyl ester of fatty acids was ready for injection into the gas chromatography (GC) machine [30]. Flame Ionization Detector (FID-GC) analysis was carried out using a Varian CP-3800 equipped with a VF-5 capillary column (fused silica, 30 m length × 0.25 mm internal diameter, film thickness 0.25 µm). Helium was used as the carrier gas at a constant flow rate of 1.1 ml min^{-1} with a split ratio of 1 : 50. The oven temperature was held at 60°C for 1 min, then programmed to increase to 250°C at a rate of 3°C min^{-1} and held for 10 min. The injector and detector (FID) temperatures were maintained at 250°C and 280°C, respectively [30].

2.3.2. Gas chromatography-mass spectroscopy

The composition of cinamon and eucalyptus oils was determined using gas chromatography-mass spectroscopy (GC-MS). The specifications and temperature settings of the device are as follows: to analyse the plant oil (PO) samples, a GC connected to a Varian 4000 mass spectrometer equipped with a DB-5 capillary column with a length of 30 m and an internal diameter of 0.25 mm was used. The oven temperature was set to increase from 60 to 250°C at a rate of 4°C min^{-1} and was maintained at 250°C for 10 min. Helium was used as a carrier gas with a flow rate of 1 ml min^{-1}, and the ionization energy was 70 eV. The mass range was set from 35 to 400, and the injection volume was 1 µl. The retention index of the spectra obtained from the essential oil was calculated using the normal inhibition time of the injected alkanes (C_{24}–C_9) under the same conditions as the essential oil. The relative percentage of each compound in the essential oil was determined based on the area under the curve in the chromatogram through normalization [30]. Compounds were identified by comparing their mass spectra with those from the internal reference mass spectra library (Wiley 7) or with authentic compounds, and their retention indices were corroborated with those of authentic compounds or those reported in the literature [31,32]. For quantification purposes, the relative area percentages obtained by the FID were used without correction factors. GC profiling of coconut, neem oil, cinnamon essential oil and eucalyptus essential oil is shown in the electronic supplementary material, figures S1–S4 and tables S1–S4.

2.3.2.1. Gas chromatography

GC analysis of coconut oil fatty acids involves separating and identifying the individual fatty acids present in the oil using GC coupled with MS. This analytical technique allows for the accurate determination of the fatty acid composition of coconut oil. The main fatty acid found in coconut oil is lauric acid, which typically comprises around 42–49% of the total fatty acids. Other major fatty acids in coconut oil include myristic acid, caprylic acid, palmitic acid, capric acid, stearic acid, elaidic acid, oleic acid, linoleaidic acid and linoleic acid.

However, the specific percentages of these fatty acids may vary depending on factors such as coconut variety, geographical location, and extraction method. During GC-MS analysis, the coconut oil sample undergoes several processes, including the extraction of fatty acids, derivatization to form volatile derivatives and separation of compounds by GC. The separated compounds are then identified and quantified using MS, which provides data on the mass-to-charge ratio of each compound. By analysing the GC-MS results, the fatty acid composition of coconut oil can be determined, enabling quality control, authenticity assessment and comparison of different coconut oil samples. This information is valuable for both research and industry purposes, helping to ensure the consistency and efficacy of coconut oil products [33].

2.3.2.2. Gas chromatography profiling of coconut oil

The chromatographic profile obtained from GC analysis is shown in the electronic supplementary material, figure S1. A total of eight compounds as detailed in the electronic supplementary material, table S1, were identified, representing 99.39% of all detected compounds; of which, eight fatty acids (99.39%) accounted for the major identified class, comprising six saturated fatty acids (SFA) (93.99%), one mono-unsaturated fatty acid (4.6%) and one polyunsaturated fatty acid (0.7%). The total SFA content was greater than that of the total unsaturated fatty acids [34].

2.3.2.3. Gas chromatography profiling of neem oil

A total of six compounds from different phytochemical classes were identified, representing 96.667% of the total detected compounds (electronic supplementary material, table S2). The chromatographic profile obtained from GC analysis is shown (electronic supplementary material, figure S2). The analysis of neem oil revealed the presence of elaidic acid and oleic acid (50.27%) [35].

2.3.2.4. Cinnamon oil (gas chromatography-mass spectroscopy)

The identified components present in the analysed cinnamon essential oil are listed in the electronic supplementary material, table S3. The chromatographic profile obtained through GC-MS analysis is shown (electronic supplementary material, figure S3). The major components in the essential oil examined were E-cinnamaldehyde, which accounts for approximately 75.353. The remaining percentage of cinnamon oil consists of small amounts of antioxidants, vitamins and minor compounds. E-cinnamaldehyde is the main component responsible for the characteristic flavour and aroma of cinnamon oil [36].

2.3.2.5. Eucalyptus oil (gas chromatography-mass spectroscopy)

The composition of eucalyptus essential oil can vary depending on the specific species of eucalyptus and the region from which it is sourced. The chromatographic profile obtained through GC-MS analysis is shown in the electronic supplementary material, figure S4. The electronic supplementary material, table S4, lists the approximate percentages of some of the main ingredients found in eucalyptus oil, as analysed by GC-MS 1,8-cineole (eucalyptol) is approximately 46.509%. Eucalyptol is the major component in many eucalyptus oils and provides the characteristic cooling, camphor-like aroma [37].

2.3.3. Rearing *of Lycoriella auripila* (Diptera: Sciaridae)

The mushroom sciarid flies were collected from the 'Protein Sefid Mushroom Company' in Tankman, near Nazarabad County, Alborz province, Iran (35.916745319704, 50.64741751316301). The sciarid flies were screened and reared on a mushroom medium in an environmental growth chamber at 25 ± 2°C, with a humidity rate of 70%, and a photoperiod of 24 hours of darkness. The infested mushroom medium was kept in a plastic container measuring 1000 mm × 600 mm for 30 days. To prevent drying, the mushroom medium was covered with black nylon that had ventilation holes. Under these conditions, the sciarid larvae could move into the medium and complete their life cycle.

2.3.4. Larvae susceptibility of *Lycoriella auripila* to the tested insecticides

For the bioassay of sciarid larvae, 150 g of infested mushroom medium (from the upper part, 50 mm thick) was placed in cylindrical containers with dimensions of 95 mm × 95 mm in diameter and height. The mushroom medium in the containers was sprayed with different concentrations of the insecticides, with water used as a control. Each concentration had seven repetitions and each infested mushroom container was sprayed with 30 ml of solution. After treatment, yellow glue cards with dimensions of 20 mm × 40 mm were placed vertically on the treated substrate to capture emerged adult flies. Finally, the containers were sealed with lids that had nets for air exchange.

The following formula was used to calculate the percentage reduction in the adult Sciaridae fly population in treatments compared to the control, based on the number of live larvae in the contaminated culture medium and the flies attracted to the sticky yellow traps: mortality (%) = $100 \times (X - Y)/X$, where X is the mean number of alive adults in the control group and Y is the number of alive adults in the treated group [38,39].

2.3.5. Bioassay of nanobioinsecticides on the mushroom Sciaridae adults

The contact bioassay was conducted using glass vials (100 mm length, 2 mm diameter), with three replicates for each insecticide concentration.

To soak the inner surfaces of the tubes, 10 ml of insecticide solution was poured into each glass vial, which was then gently swirled until the inner surfaces were uniformly soaked. The vials were left at laboratory temperature to dry. Ten adult Sciaridae flies were collected using an aspirator and released into the glass vials. The doors of the tubes were covered by a cloth net to allow for ventilation. The number of live Sciaridae flies in each test tube was counted 24 h after treatment. For the dose–response bioassays, the insecticides were tested at four concentrations: 750, 1500, 2250 and 3000 mg a.i. l^{-1}. These concentrations were selected to cover the expected range of activity and to allow accurate estimation of LC_{50} values.

2.4. Statistical analysis

The mortality data of the developmental stages of Sciaridae were transformed using a square-root transformation to normalize variances. The data were then analysed with analysis of variance (ANOVA). When *F*-values were significant ($p \leq 0.05$), the mean treatments were compared using the Duncan multiple range test (SAS v. 9.4, SAS Institute, 2020). The LC_{50} values of the nanobioinsecticide and chemical insecticides were calculated by PROC PROBIT [40] in SAS v. 9.4. LC_{50} values and their 95% confidence intervals were compared using the overlap method; non-overlapping intervals indicated significant differences in toxicity among the insecticides.

3. Results

3.1. Synthesis and characterization of nanobioinsecticide

Nanobioinsecticidal formulations based on essential oils were prepared using a two-step oil-in-water (O/W) emulsification method. The hydrophilic–lipophilic balance (HLB) value of the nanoemulsion was determined to be between 13 and 15, which is optimal for stable O/W emulsions. Based on HLB calculations and preliminary stability tests, a stable formulation was selected for further analysis. The final nanoemulsion formulation consisted of 15% (v/v) essential oil as the oil phase, 3% (v/v) acetone as a co-solvent and 75.88% (v/v) Tween 80 which corresponds to 15% (v/v) of the total emulsion volume, used as the primary surfactant. The oil-phase components were mixed vigorously for 2 h to ensure homogeneity. Subsequently, the organic phase was slowly added to the aqueous phase under continuous stirring to form a coarse emulsion, which was then subjected to high-speed homogenization to produce a transparent nanoemulsion.

The resulting nanoemulsion was stored in dark glass bottles at an ambient temperature (25 ± 2°C), and its physcochemical stability was evaluated over a period of three consecutive months [41]. Visual observations, including colour change, phase separation, creaming and sedimentation, confirmed that the formulation remained clear and homogeneous throughout the storage period. Additionally, the aqueous solution derived from the stable nanoemulsion exhibited good long-term stability when tested over a minimum of three months.

The pH of the final formulations ranged from 5.03 to 7.20, indicating acceptable compatibility for potential agricultural and environmental applications. Particle size analysis, performed using DLS, revealed that the nanobioinsecticide had an average particle size in the range of 10–30 nm. Stability assessments were conducted on both freshly prepared samples (figure 1a) and samples stored for 30 days (figure 1b), confirming no significant changes in particle size or polydispersity index. Morphological characterization of the nanoemulsion was carried out using TEM and SEM. TEM images (figure 1c) confirmed the DLS results, showing uniform and predominantly spherical nanoparticles. SEM analysis (figure 1d) further verified the spherical morphology of the particles, with an average size of approximately 50 nm. These results indicated a monodisperse distribution and consistent particle shape, supporting the formation of a stable and well-structured nanobioinsecticide [42].

3.2. Evaluation of nanobioinsecticide for pest control

3.2.1. Susceptibility of Sciaridae flies developmental stages (larvae)

The results indicated that these compounds could effectively control the Sciaridae fly population (table 1). The high concentration of nanobioinsecticide demonstrated the highest efficacy, ranging from 92% to 100% against larval stages of flies 7–14 days after spraying, and was ranked in group A. By contrast, the chemical insecticides such as thiamethoxam and imidacloprid exhibited an efficacy range of 52–93% during the two weeks following treatment (table 1). Subsequent evaluation showed that the effectiveness of the treatments increased, and by 23 days after spraying, the efficacy levels were not significantly different, with all treatments achieving an effectiveness range of 98–100% (table 1).

Evaluation of live adults of *L. auricula* captured on yellow cards indicated that the high-concentration nanobioinsecticide and imidacloprid insecticide were the most effective against Sciaridae flies (figure 2a–c). The low-concentration nanobioinsecticide (67%) and thiamethoxam (62%) treatments demonstrated the lowest efficacy, placing them in the low effectiveness group. All tested treatments

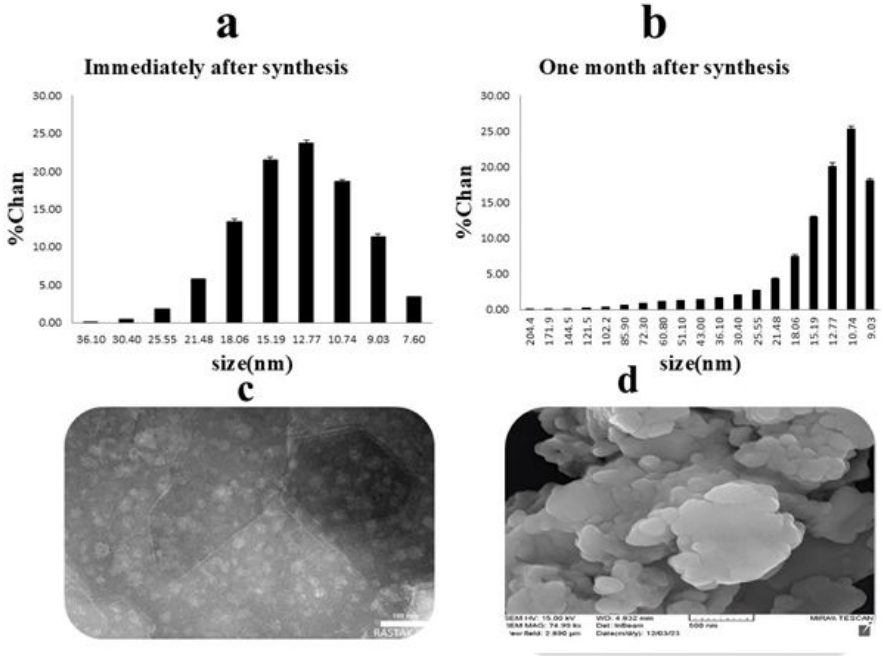

Figure 1. Characterization of the nanobiopesticide. (a) Dynamic light scattering (DLS) image of the as-prepared formulation. (b) DLS image taken 30 days after preparation. (c) Transmission electron microscopy (TEM) micrograph. (d) Scanning electron microscopy (SEM) micrograph of the nanobiopesticide.

exhibited reduced efficacy during the first seven days after treatment; however, evaluations conducted two weeks after treatment revealed an increasing trend in efficacy across all treatments (table 2). According to Duncan's test at the 5% significance level, the mean efficiency percentages for the nanobioinsecticide and imidacloprid treatments reached 100%, categorizing them in the high efficacy group. Conversely, the mean efficacy of thiamethoxam and the low-concentration nanobioinsecticide remained below 100%, thus placing them in the second group (table 2).

3.2.2. Bioassay on adult sciarid flies

We evaluated the toxicity of the nanobioinsecticide (figure 3a) against adult Sciarid flies (figure 3b,c). As shown in table 3, the nanobioinsecticide exhibited insecticidal activity against adult Sciarid, with an LC_{50} value of 765.97 (283.2–2550.15 mg a.i. l^{-1}). The results indicated that the adult Sciarid fly is most susceptible to imidacloprid, which had an LC_{50} value of 116.4 mg a.i. l^{-1}, while thiamethoxam had an LC_{50} = 201.08 mg a.i. l^{-1} (table 3). There is no significant difference between the two chemical insecticides (table 3).

4. Discussion

POs possess diverse and complex chemical compositions and have been used for pest control. However, their use is limited owing to volatility, poor water solubility and environmental instability [43]. The protective effects of nanocarriers and nanoformulations for unstable active ingredients have been reported against environmental factors, including pH, oxygen and light. These nanocarriers act as a barrier between the bioactive compounds and environmental factors [44]. Today, the synthesis of nanoformulation of various plant-derived compounds is of growing interest, especially the nanoemulsion of O/W types are promising [45]. In our study, the results of bioassay tests on larvae and adult sciarid flies showed that nanobioinsecticides, like chemical insecticides, can effectively control edible mushroom pests. Additionally, the nanoformulation is effective in increasing the stability of plant extracts and oils. Furthermore, using a mixture of several organic insecticides in a nanoformulation can enhance the effectiveness of organic pesticides (tables 1–3).

Table 1. Mean mortality percentage of developmental stages of Sciaridae fly based on the alive larvae on days after treatment, determined by Duncan's test. (Similar letters in each row are not significantly different. Different concentrations of nanobioinsecticide, nano-*E*: $E_{750} = 750$, $E_{1500} = 1500$, $E_{2250} = 2250$, $E_{3000} = 3000$ mg a.i. l^{-1} and traditional insecticide: imidacloprid = 175 mg a.i. l^{-1}, thiamethoxam = 125 mg a.i. l^{-1}.)

			nanobiopesticide treatments							
days	thiamethoxam 250WG	imidacloprid 350SC	nano-E_{750}	nano-E_{1500}	nano-E_{2250}	nano-E_{3000}	d.f.	F_{value}	p	CV (Coefficient of Variations)
+7	52.22 ± 4.15c	71.11 ± 3.23b	61.11 ± 3.92bc	66.66 ± 3.92b	83.33 ± 2.48a	92.22 ± 2.83a	5.24	17.65	<0.0001	10.95
+15	88.0 ± 2.09c	93.14 ± 1.45bc	88.57 ± 2.02c	92.0 ± 1.66bc	96.0 ± 1.45ab	98.85 ± 0.70a	5.24	6.64	0.0005	3.93
+20	95.51 ± 0.70cd	98.65 ± 0.54ab	95.96 ± 0.83cd	97.76 ± 0.70bc	99.10 ± 0.54ab	100 ± 0a	5.24	8.22	0.0001	1.41
+23	98.87 ± 0.75a	100 ± 0a	99.24 ± 0.46a	99.62 ± 0.37a	100 ± 0a	100 ± 0a	5.24	1.48	0.2342	0.87



Figure 2. Field and laboratory contexts of the study. (a) Adult Sciaridae fungus gnats. (b) Greenhouse cultivation of edible mushrooms. (c) Experimental container used for larval bioassays of Sciaridae fungus gnat larvae.

Spraying edible mushroom beds with nanobioinsecticide, imidacloprid and thiamethoxam showed that a high concentration of nanobioinsecticide (90%) was more effective in the first two weeks. By contrast, lower concentrations were as effective as both chemical insecticides, achieving an efficiency of 61–66%. The high efficiency of the nanobioinsecticide at elevated concentrations may be attributed to the plant essential oils in the nanoformulation, which enhance the insect's inhalation toxicity. The increase in efficiency across all treatments could be owing to the stomach toxicity of each insecticide and their stability under the conditions of the mushroom bed (pH = 5.5–5.9, dark, 14–16.5°C). The tested beds were placed in closed containers preventing adult insects from entering and laying eggs. Ultimately, all sciarids died because of respiratory, digestive and contact toxicity within the container. The eggs in the cultivation bed also gradually hatched and then died. In this experiment, the toxicity of treatments on the egg and pupal stages were not examined, which may be a limitation of this research.

A study conducted by Jess *et al.* [45] demonstrated the control of *Lycoriella ingenua* (Diptera: Sciaridae) and *Megaselia halterata* (Diptera: Phoridae), *two* pests in edible mushrooms, using garlic essential oil as a repellent. They treated compost with various concentrations of garlic essential oil (0.1–20%), which successfully repelled adult *M. halterata* flies. The highest concentration of garlic essential oil (20%) significantly reduced adult emergence from treated compost in the case of *L. ingenua*.

The contact toxicity of insecticides on adult sciarids showed that the efficacy of chemical insecticides is greater than that of nanobioinsecticides. This difference is significant, as shown by the pairwise comparison method lethal dose ratio $(LDR)_{50}$; specifically, imidacloprid and thiamethoxam were found to be 6.5- and 3.8-fold more toxic than the nanobioinsecticide (table 3; figure 4). There was no significant difference between the two chemical insecticides, imidacloprid exhibited greater contact toxicity against adult sciarids. A study on the toxicity of three insecticides including imidacloprid, diazinon and deltamethrin against *L. auripila* found that imidacloprid was the most effective, providing 100% control at high concentrations. By contrast, low concentrations of diazinon actually increased the emergence of adults, while deltamethrin proved ineffective against *L. auripila* [46].

Researchers have also reported the insecticidal activity of POs and their constituents against *L. ingenua*. Essential oils such as black cumin (*Carum carvi* L.), lemon grass (*Cymbopogon citratus* D.C. Stapf), mandarin (*Citrus reticulata* Blanco) and nutmeg showed good activity (>90%) against *L. ingenua* larvae [47]. Jaffar & Lu [48] demonstrated the toxicity, oviposition inhibition and repellent activity of six major essential oil compounds against both adult and immature stages of the oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae). In another study, an azadirachtin-based insecticide (Ozoneem Trishul 1%) was evaluated for controlling the fungus gnat *L. ingenua*. Its efficacy was compared with that of malathion, a conventional insecticide. The results showed that the azadirachtin-based formulation successfully reduced the abundance of fungus fly [49].

Essential oils exert insecticidal effects through various biochemical pathways. Key components, including terpenoids like citronellal, 1,8-cineole, geraniol and eugenol, disrupt the insect nervous system by modulating octopaminergic receptors, interfering with GABA-gated chloride channels and inhibiting acetylcholinesterase activity [50]. This leads to paralysis, impaired respiration and feeding inhibition. Additionally, phenolic compounds increase membrane permeability, causing leakage of cellular contents and metabolic dysfunction [51]. In sciarid flies, these mechanisms may enhance both contact and inhalation toxicity, especially in confined environments such as mushroom cultivation rooms.

Table 2. Mean mortality percentage of developmental stages of Sciaridae fly based on the alive adults on days after treatment in tested insecticides, determined by Duncan's test. (Similar letters in each row are not significantly different. Different concentrations of nanobioinsecticide, nano-*E*: $E_{750} = 750$, $E_{1500} = 1500$, $E_{2250} = 2250$, $E_{3000} = 3000$ mg a.i. l^{-1} and traditional insecticide: imidacloprid = 175 mg a.i. l^{-1}, thiamethoxam = 125 mg a.i. l^{-1}.)

			nanobiopesticide treatments							
days	thiamethoxam 250WG	imidacloprid 350SC	nano-E_{750}	nano-E_{1500}	nano-E_{2250}	nano-E_{3000}	d.f.	F_{value}	p	CV (Coefficient of Variations)
+7	62.40 ± 2.65c	71.42 ± 2.54ab	67.67 ± 1.91bc	73.68 ± 2.65ab	75.18 ± 3.48ab	81.20 ± 3.36a	5.24	5.24	0.0022	8.76
+15	82.25 ± 1.32b	87.57 ± 1.72a	81.06 ± 1.50b	87.57 ± 1.72a	89.94 ± 1.77a	92.30 ± 1.18a	5.24	7.85	0.0022	4.0
+20	92.50 ± 1.11b	98.5 ± 1.0a	91.0 ± 1.69b	98.0 ± 0.93a	99.0 ± 0.61a	100 ± 0a	5.24	13.36	<0.0001	2.38
+23	97.78 ± 0.7b	100 ± 0a	96.0 ± 1.29c	100 ± 0a	100 ± 0a	100 ± 0a	5.24	7.99	0.0001	1.36

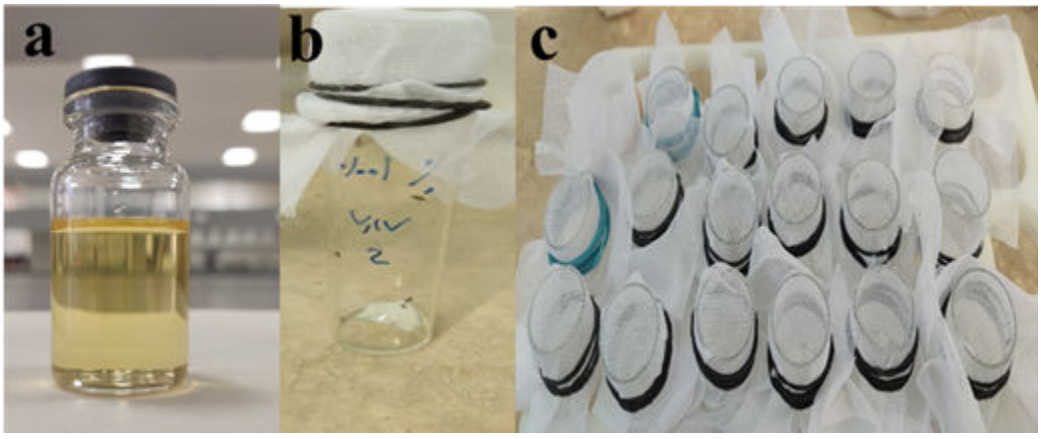

Figure 3. Laboratory assays were conducted in the study. (a) The prepared nanobiopesticide formulation. (b,c) Adult bioassay tests performed on Sciaridae fungus gnat adults.

Table 3. Bioassay (LC_{50}) of tested insecticides and their parameters against sciarid flies at 24 hours after treatment. (If the 95% confidence interval of lethal dose ratios includes 1, then the LDs are not significantly different.)

treatment	d.f.	LC_{50}[a] (low–up) 95% CI	slope ± s.e.	χ^2[b]	lethal dose ratio (LDR_{50})	toxicity rate
nanobioinsecticide	4	765.97 (283.2–2550.15)	0.54 ± 0.123	0.256	1	1
thiamethoxam	4	201.08 (115.90–380.05)	1.013 ± 0.251	0.633	2.052–19.640	3.8
imidachlopyride	4	116.41 (73.82–176.18)	1.324 ± 0.256	0.935	5.218–45.174	6.5

[a]LC_{50} (mg a.i. l^{-1}), lethal concentration for 50% mortality with 95% confidence limit; LCL, lower confidence limit; UCL, upper confidence limit.

[b]χ^2, chi-square value with $\alpha = 0.05$; s.e., standard error.

Synergistic interactions among essential oil constituents may further explain the high efficacy of the nanobioinsecticide used in our study. Combinations of terpenoids and phenolics can enhance penetration through the insect cuticle, prolong persistence and intensify neurotoxic effects [52]. For example, citronellal and eucalyptol exhibit synergistic fumigant toxicity, while mixtures containing azadirachtin can inhibit detoxification enzymes, increasing susceptibility to other components [53]. However, some antagonistic interactions may occur when compounds compete for the same metabolic pathways or receptor targets, potentially reducing overall toxicity [54]. The nanoformulation in our study probably promotes synergy by stabilizing volatile constituents, enhancing dispersion and ensuring simultaneous delivery of active molecules to target sites. This may explain why lower concentrations of the nanobioinsecticide achieved toxicity levels comparable to those of chemical insecticides.

The use of pesticide mixtures against pests is often employed to enhance their effectiveness and to address the limitations of some active ingredients. In recent years, research on binary organic pesticides has been limited; however, it is anticipated that there will be an increase in binary organic insecticide mixtures in the coming years. The contact toxicity of citronella and eucalyptus essential oils, when used alone on houseflies, has been reported to increase mortality within 20–40% at 6–24 h after exposure [55]. Based on this information, we selected both insecticides for testing on mushroom flies. Our experiment demonstrated that nanobioinsecticides are as effective as chemical insecticides. Additionally, a 5% extract of azadirachtin applied to edible mushroom cultivation medium significantly reduced sciarid infestation severity and increased the yield of edible mushrooms compared to the control [56].

Studies evaluating nanoformulation of plant essential oils indicate that nano-encapsulation enhances synergistic effects by improving solubility, protecting unstable molecules and facilitating

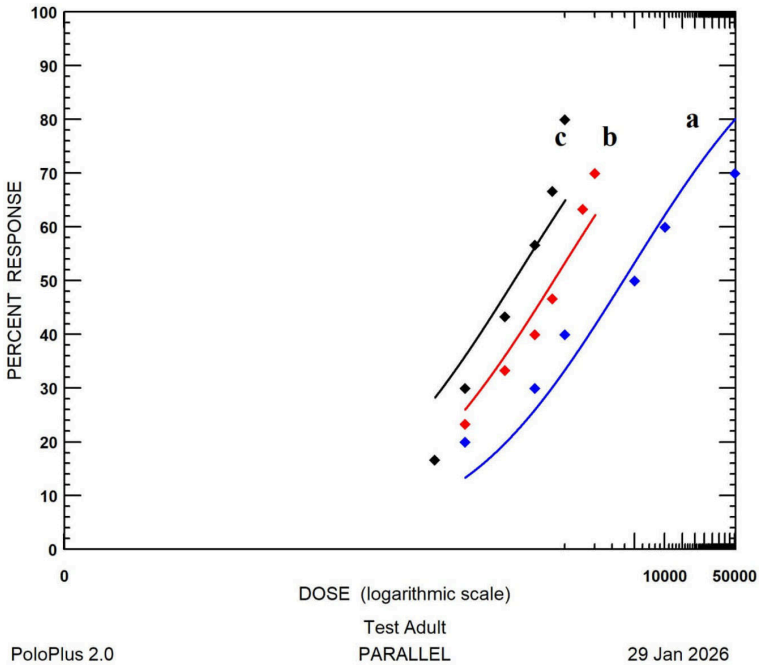

Figure 4. Biometric profiles of Diptera: Sciaridae adults treated with different insecticides: **a** nanobiopesticide formulation, **b** thiamethoxam, and **c** imidacloprid. Measurements illustrate morphological responses under each treatment.

controlled release [57]. This sustained release profile maintains effective concentrations over extended periods, which is particularly advantageous in mushroom beds, where humidity and temperature can degrade conventional formulations. Furthermore, nano-sized droplets increase surface contact and penetration efficiency, further amplifying the synergistic interactions among active constituents.

Studies have shown that nanoformulations of plant essential oils and extracts as a mixture can increase their insecticidal effects and prolong their duration of efficacy [58]. The application of a mixture of plant extracts and essential oils on *Pistachio psylla* showed that the combination of azadirachtin, eucalyptus and chamomile is effective on *P. psylla* [59].

Nanobioinsecticides and plant essential oil formulations have been shown to exhibit low toxicity towards non-target organisms, including mammals, birds and aquatic species, which supports their environmental safety [60]. Toxicity assays indicate minimal acute and chronic effects at concentrations effective against sciarid pests, confirming that these formulations can be safely applied in edible mushroom cultivation with a reduced risk to humans and the environment [55].

Although nanoformulation of plant insecticides has not yet been registered for use against sciarids in edible mushrooms, recent research has focused on chitosan, plant extracts and their integration with biological control agents as environmentally friendly alternatives to chemical control methods [61].

5. Conclusion

In summary, a novel nanobioinsecticide has been developed as a nanoemulsion-based formulation that incorporates neem and coconut oils, along with essential oils of eucalyptus, cinnamon and citronella. This eco-friendly insecticide demonstrates high efficacy against sciarid flies while remaining non-toxic to plants, humans and the environment—posing no harm to soil or subsurface water. Compared to conventional chemical insecticides, it offers significant advantages, including biodegradability, environmental safety and compatibility with integrated pest management systems in mushroom cultivation. These findings provide valuable insights for researchers and farmers aiming to advance biopesticide development, supporting the creation of more targeted, efficient and sustainable pest control strategies—especially for sensitive agricultural settings like edible mushroom production.

Ethics. This work did not require ethical approval from a human subject or animal welfare committee.

Data accessibility. The authors confirm that the data supporting the findings of this study are available within the article and/or its Electronically Stored Information (ESI) [62]. An Excel file has been provided as electronic supplementary material. This file contains the raw data corresponding to tables 1–3. Each table is organized in

a separate sheet: sheet 1 includes the data for table 1; sheet 2 for table 2; and sheet 3 for table 3. This electronic supplementary material has been prepared to ensure transparency and to facilitate the review of the study's results.

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. M.N.-N.: formal analysis, methodology; M.G.: conceptualization, data curation, investigation, methodology, project administration, supervision, writing—original draft, writing—review and editing; L.M.: investigation, methodology, project administration, resources, supervision, writing—original draft, writing—review and editing; A.S.: data curation, investigation, software, writing—original draft; M.A.H.: formal analysis, resources.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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