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Extraction, Purification of Avermectin from the Iraqi *Streptomyces avermitilis* Strain Amna-4 and Evaluation of its Efficacy in Killing *Megaselia halterata* Parasitizing the Edible Mushroom *Pleurotus pulmonarius*

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ABSTRACT

Background: Oyster mushroom, *Pleurotus* spp. are considered healthy foods with high nutritional value. This mushroom is exposed to many pests, including *Megaselia halterata* infestation, which causes it to lose its quantity and quality. The current study was conducted to evaluate the efficacy of Avermectin from *Streptomyces avermitilis* in controlling *M. halterata*

Methods: Avermectin was partially purified from *S. avermitilis* and its quantity and quality were determined using HPLC. Standard conditions were calibrated for maximum productivity and its effectiveness against *M. halterata* was tested in the mushroom growing room.

Result: The results showed that the insect was identified as *M. halterata* through molecular diagnosis based on nucleotide sequence analysis of the gene cytochrome oxidase C subunit 1 and it was registered on the NCBI website under the name *M. halterata* voucher Amna-1 (accession number PP320397.1). Results of the partially purified avermectin from *S. avermitilis* strain Amna-4 analyzed by HPLC showed a retention time of 12.5 minutes, close to the standard avermectin retention time of 12.4 minutes. The optimal conditions for avermectin production from *S. avermitilis* were identified, with the highest concentration recorded at 342.59 mg/L in SCM (Starch Casein Medium) supplemented with 5% carboxymethyl cellulose and 5% isoleucine at a pH of 7.5, incubated at 30°C for 14 days. When evaluating the efficiency of the partially purified avermectin in infecting *P. pulmonarius* with *M. halterata*, the results showed no insect infestation comparable to the commercial insecticide Proclaim and the uninfected control treatment. The biological efficiency was recorded as 101.27, 99.72 and 98.86% for the partially purified avermectin, Proclaim and the healthy uninfected control, respectively. The results also demonstrated a clear effect on the fruit bodies' protein content in the *M. halterata* treatment only, where it decreased to 18.51%, while no significant differences were observed among the partially purified avermectin, Proclaimÿþ and the uninfected control treatments, which reached 35.21, 34.78 and 34.59%, respectively.

Key words: Avermectin, *Megaselia halterata,* Oyster mushroom, *Pleurotus pulmonarius*, *Streptomyces avermitilis*.

INTRODUCTION

The oyster mushroom *Pleurotus* spp. is a widely recognized edible fungus valued for its rich nutritional profile, including high-quality proteins, essential vitamins and minerals, alongside notable antioxidant and therapeutic properties that benefit human health (Iqbal *et al*., 2024). Oyster mushroom extracts are also important in inhibiting some types of pathogenic bacteria (To Hong *et al*., 2023). On an environmental level, oyster mushrooms are cultivated on various agricultural wastes, thus contributing to resource recycling (Getachew and Abebe, 2021; Karpagavalli *et al*., 2025). Mushroom cultivation continuously faces challenges from various insect pests, particularly mushroom flies such as those from the genera *Lycoriella, Bradysia* and *Megaselia*, which infest all stages of mushroom production-from substrate colonization to fruit body development. These flies have been confirmed as major pests attacking commercially cultivated species including *Pleurotus* spp., *Agaricus* spp. and *Lentinus* spp. and other mushrooms, causing significant quantitative and qualitative losses (Pandey *et al*., 2025; Shikano *et al*., 2021). The damage inflicted by mushroom flies is twofold: larvae feed directly on developing fruit bodies and fungal mycelium within the substrate, thereby reducing nutrient availability, while concurrently acting as vectors for microbial pathogens such as bacteria, fungi, viruses and nematodes. Even low larval densities can lead to considerable yield declines (White, 1985). Among these pests, the mushroom phorid fly *M. halterata* remains the most pervasive species globally,

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having been extensively documented since its first large-scale outbreak in British farms in 1953 (Grewal, 2007; Shikano *et al*., 2021). In Iraq, insect infestation in cultivated mushrooms was identified by Hassan and Al-Qaissi (2022) at the mushroom farm, College of Agriculture, Tikrit University. They observed damage symptoms such as holes, necrosis and tunnels in fruit bodies of *Agaricus bisporus* and *Pleurotus* spp. Morphological identification was confirmed through molecular diagnosis using mitochondrial cytochrome oxidase subunit 1 (COI) sequencing, which detected the presence of *M. halterata* (MZ021516.1) and *Lycoriella ingénue* (MZ021517.1) (Hassan and Al-Qaissi, 2022).

Ongoing integrated pest management (IPM) research focuses on safe, multifaceted approaches to control these flies, including physical exclusion, biological control and optimized environmental management, aiming to reduce reliance on chemical pesticides and mitigate pest pressures in major mushroom-producing regions (Pandey *et al*., 2025). Previous studies have demonstrated the importance of biological control in controlling many oyster mushroom pathogens and pests (Rakhmonov and Soatov, 2023). The filamentous bacterial related to *Streptomyces* is renowned for producing more than 70% of clinically and agriculturally important antibiotics and secondary metabolites (Lee *et al*., 2021). Several *Streptomyces* species isolated from soil demonstrate strong insecticidal properties against pests like aphids and red spider mites, along with antibacterial and antifungal effects against plant pathogens (Khan *et al*., 2023). Various secondary metabolites from *Streptomyces* spp. have been identified as potent insecticidal agents (Amelia-yap *et al*., 2023). Among these, *S. avermitilis* plays a crucial role due to its production of avermectins, macrocyclic lactones that disrupt insect nervous systems by modulating chloride channels, leading to paralysis and death (Cerna-Chávez *et al*., 2024). Avermectin B1a, the most effective homolog, is widely used in agriculture as a safe biopesticide with minimal risk to non-target organisms and the environment (Du *et al*., 2025).

Due to the importance of oyster mushrooms as a balanced and healthy food source and their exposure to significant losses caused by insect infestation, as well as the critical role of using biological agents and their products as safer alternatives to chemical pesticides against insects, this study was conducted. It aims to extract, purify and determine the optimal conditions for the production of avermectin from *S. avermitilis* and to evaluate its efficacy in reducing insect infestation.

MATERIALS AND METHODS

This study was conducted in the laboratories of the pioneering mushroom production farm at the College of Agriculture, Tikrit University, Iraq during the period 2024-2025.

Streptomyces avermitilis

The Iraqi bacterial strain *S. avermitilis* strain Amna-4, molecularly identified (NCBI accession number: PP320403.1), was obtained from a previous study (Shaker and Hassan, 2025).

Culture media

The following media were specially used for actinomycete bacterial cultivation.

Starch csein medium (SCM): (Bawazir *et al*., 2023), glycerol asparagine medium (GAM): (Fair and Tor, 2014), yeast extract-malt extract medium (YMM) (Shepherd *et al*., 2018), starch yeast extract medium (SYM) (Collins *et al*., 1995), glycerol tyrosine medium (GTM) (Nawani, 2002), glycerol yeast extract medium (GYEM) (Collins *et al*., 1995), starch minerals medium (SMM) (Williams *et al*., 1983) and starch peptone yeast extract medium (SPYM) (Collins *et al*., 1995).

The media were evenly distributed into five 250 ml glass flasks, each containing 200 ml. The flasks were tightly closed with cotton plugs and sterilized in an autoclave at 121°C and 1.5 kg/cm² pressure for 15 minutes.

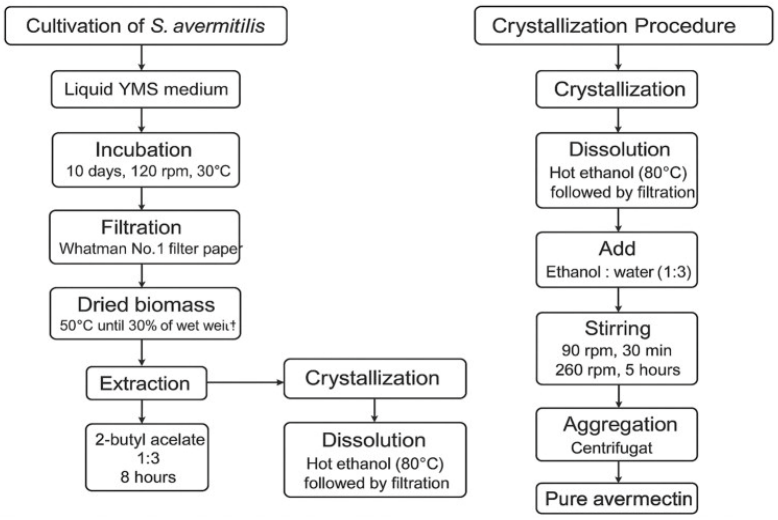


Fig 1: Flow-diagrams summarized the extraction and crystallization of avermectin from *S. avermitilis*.

Cultivation of *S. avermitilis* and extraction and purification of avermectin

Avermectin was extracted and purified from *S. avermitilis* strain Amna-4 according to the method described by Xu (2012). The bacterium was cultivated in liquid YMS medium with an incubation period of 10 days in a shaking incubator at 120 rpm and 30°C. Filtration was performed using Whatman No. 1 filter paper to obtain the biomass. The biomass was dried in an electric oven at 50°C until reaching 30% of its wet weight. Extraction was carried out with 2-butyl acetate (1:3 ratio; 100 g dry biomass to 400 ml 2-butyl acetate) for 8 hours. After filtration, the residual biomass was subjected to a second extraction under the same conditions. The combined filtrate, called the organic ester phase extraction liquid, underwent a washing step to remove impurities using a 5% solution of tetrabutyl ammonium bromide (organic ester phase to wash solution ratio 4:1). The upper layer containing impurities was discarded and the precipitate was used in the crystallization step.

Crystallization step

The mixture was left for 10 hours to allow crystal formation. Once crystals formed, they were dissolved in hot ethanol at 80°C and then filtered as the ethanol temperature decreased to 22°C. An ethanol:water mixture (1:3) was added to the previous mixture and stirred in a shaking incubator at 90 rpm for 30 minutes, followed by rapid stirring at 260 rpm for 5 hours. This change in stirring speed promoted crystallization. Subsequently, formed aggregates were collected by centrifugation, concentrated by rotary evaporation and dried to obtain pure avermectin powder. Fig 1 summarized the extraction and crystallization of avermectin from *S. avermitilis.*

Quantitative and qualitative estimation of partially purified avermectin using HPLC

Avermectin was quantified following the method of Siddique *et al*. (2014). Fifty milligrams of avermectin from this study were dissolved in 20 ml of 96% methanol and the volume was completed to 50 ml with deionized distilled water. A 50 µL aliquot of the sample was injected into an HPLC system (Shimadzu LC-10A) using methanol:acetonitrile (98:2 v/v) as the mobile phase, Column,C-18, dimensions 50 × 4.6 mm, Flow rate,0.5 mL/min, Detector type,UV-VIS detector at 245 nm. Avermectin concentration was calculated using the standard avermectin (Sigma, USA):

Optimization of avermectin production conditions from intracellular extract of *S. avermitilis*

Determination of the optimal medium

Eight media previously used for actinomycete cultivation were tested, with pH adjusted to 8. The sterilized media were inoculated with three cork-borer plugs (0.5 cm diameter) from a 12-day-old bacterial colony and incubated at 28°C for 12 days.

Determination of optimal pH

The medium with the highest avermectin production (SCM) was adjusted to pH values of 6 to 9 using 0.1 M NaOH and 0.1 N HCl. The media were inoculated and incubated under the previously described conditions.

Determination of optimal temperature

The highest producing medium (SCM) with pH adjusted to 7.5 was incubated for 12 days at temperatures of 24, 26, 28, 30 and 32°C.

Determination of best carbon sources

Carbon sources (5%) including mannitol, dextrose, fructose, sucrose, glycerol and carboxymethyl cellulose (CMC) were added to the SCM at pH 7.5, incubated for 14 days at 30°C.

Determination of best nitrogen sources

Nitrogen sources (5%) including peptone, sodium nitrate, ammonium bicarbonate, ammonium chloride, valine and isoleucine were tested in SCM with pH 7.5 and 5% CMC, incubated for 14 days at 30°C.

Molecular identification of the insect

The insect was identified to the species level using molecular diagnosis based on the nucleotide sequence of the mitochondrial cytochrome oxidase subunit I gene (COI). Extraction of genomic DNA, PCR and sequencing were analyzed according to Folmer *et al.* (1994).

Evaluation of partially purified avermectin efficacy

This experiment was conducted at the edible fungi production farm, College of Agriculture, University of Tikrit, in a controlled environment growth chamber. The standard method for cultivation and fruiting body production of *P. pulmonarius* was employed using bags measuring 30 × 20 cm (Hassan and Muhammad, 2023). The experiment involved releasing 400-500 adult *M. halterata* insects into the mushroom growth chamber. Treatments included partially purified avermectin, Proclaim insecticide (active ingredient: emamectin benzoate, Syngenta Company) as a positive control, negative controls; (no insect infestation and no chemical addition by isolating mushroom bags in a plastic box covered with double layers of gauze) and an insect-only treatment, with five bags per treatment. The bags were incubated at 24°C, 75% relative humidity and a photoperiod of 12 hours light/12 hours dark using cool white fluorescent lamps. When primordia appeared, 100 ml of each insecticide (0.2%) was sprayed per bag.

Effect of partially purified avermectin on *P. pulmonarius* infestation by *M. halterata*

Upon fruiting body maturity, the infestation percentage was calculated as:

$$\text{Infestation (\%)} = \frac{\text{Number of fruit bodies infested with larvae}}{\text{Total number of fruit bodies}} \times 100$$

The number of larvae per 100 g of infested fruit bodies was also recorded.

Effect of partially purified avermectin on *P. pulmonarius* productivity measured by biological efficiency

Biological efficiency was calculated as: (Hassan and Muhammad, 2023).

Biological efficiency =

$$\frac{\text{Fresh weight of fruit bodies}}{\text{Dry weight of cultivated substrate}} \times 100$$

Effect of partially purified avermectin on protein content in fruit bodies of *P. pulmonarius*

Protein content was determined according to the AOAC (2022) methodology.

Statistical analysis

Experiments were conducted following a completely randomized design (CRD). Variance analysis was performed using SPSS software and means were compared by least significant difference (LSD) test at a 0.05 significance level.

RESULTS AND DISCUSSION

Molecular identification of the insect *Megaselia halterata*

Fig 2 shows the electrophoretic separation of the PCR product. A band corresponding to the PCR amplification of the mitochondrial cytochrome oxidase subunit I gene (COI) with a molecular size of approximately 720 base pairs was observed, confirming that the samples belong to insects (Folmer *et al*., 1994).

Table 1 presents sequence alignment results showing 100% identity between the COI gene sequences of the insect under study and four *M. halterata* isolates from Pakistan registered under accession numbers KY846734.1, KY838691.1, KY846190.1 and KY841049.1.

Quantitative and qualitative analysis of avermectin from the iraqi isolate *S. avermitilis* strain Amna-4

HPLC analysis of partially purified avermectin from *S. avermitilis* strain Amna-4 (Fig 3) showed a retention time of 12.5 minutes, closely matching the standard avermectin retention time of 12.4 minutes. The avermectin concentration was measured at 44.76 µg/mL.

Optimum conditions for avermectin production

Nutrient media

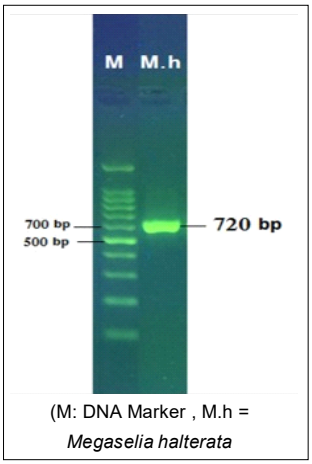
Fig 4 illustrates the effect of different nutrient media on avermectin production from the intracellular extract of *S. avermitilis* under pH 8, incubation temperature of 28°C and incubation period of 12 days. Results showed that the highest avermectin concentrations were 271.45 mg/L and 255.09 mg/L in SCM and YMM media, respectively.

pH Effect

Fig 5 shows the effect of pH values on avermectin production. The highest concentration of 282.16 mg/L was recorded at pH 7.5 in the optimal SCM medium at 28°C after 12 days incubation, followed by 265.81 mg/L and 272.33 mg/L at pH 7 and 8, respectively. Extreme pH values of 6, 6.5 and 9 resulted in the lowest avermectin yields.

Temperature

Fig 6 shows the effect of incubation temperature on avermectin production by *S. avermitilis* cultured in SCM



(M: DNA Marker , M.h = *Megaselia halterata*

Fig 2: Electrophoresis of the PCR product - 2% agarose gel concentration.

Table 1: Molecular identification of *Megaselia halterata* Voucher Amna-1 (NCBI accession no. PP320397.1) based on percentage I dentity of cytochrome c oxidase subunit I (COX1) sequences compared with selected registered strains in the global genetic database.

Insects sp./ strain	Accession No.	Country	Similarity %
Megaselia halterata voucher BIOUG02539-A09	KY846734.1	Pakistan	100
Megaselia halterata voucher BIOUG04902-E10	KY838691.1	Pakistan	100
Megaselia halterata voucher BIOUG04901-B04	KY846190.1	Pakistan	100
Megaselia halterata voucher BIOUG05383-E08	KY841049.1	Pakistan	100
Megaselia halterata voucher BIOUG05257-A10	KY838340.1	Pakistan	99.81
Megaselia halterata isolate Kb18_2019	MW807241.1	Hungary	99.81
Megaselia halterata voucher BIOUG32610-G07	MG295856.1	Canada	99.63
Megaselia halterata voucher BIOUG08684-B10	KR763612.1	Canada	99.61
Megaselia halterata voucher BIOUG32312-A10	MG103798.1	Canada	99.44

medium at pH 7.5 and 12 days incubation. The highest avermectin concentration of 296.12 mg/L was recorded at 30°C, followed by 281.55 mg/L at 28°C.

Carbon sources

Fig 7 illustrates the impact of 5% carbon sources in SCM medium at pH 7.5, with 14 days incubation at 30°C. The highest avermectin concentration of 318.38 mg/L was obtained with the addition of carboxymethyl cellulose (CMC), followed by 314.06 mg/L when 5% glycerol was added. The lowest avermectin concentration, 287.75 mg/L, was observed with mannitol as the carbon source.

Nitrogen sources

The results presented in Fig 8 demonstrate the effect of nitrogen sources at 5% concentration on avermectin production in SCM medium at pH 7.5, 14 days incubation at 30°C, with 5% CMC as the carbon source. The highest avermectin concentrations of 342.59 mg/L and 338.17 mg/L were achieved with isoleucine and valine as nitrogen sources, respectively. The addition of ammonium chloride, ammonium sulfate and sodium nitrate resulted in the lowest avermectin concentrations.

Efficacy of partially purified avermectin

The results (Fig 9) demonstrated a significant positive effect of partially purified avermectin from *S. avermitilis* in reducing the infestation rate of *M. halterata* to zero, comparable to the commercial insecticide Proclaim and the uninfested control. The infestation rate in the insect-only treatment reached 79.87%. No larvae were recorded in the avermectin and proclaim treatments, whereas 53.46 larvae per 100 g of *P. pulmonarius* fruiting bodies were counted in the insect-only treatment. Regarding fruiting body productivity of *P. pulmonarius*, the biological efficiency percentages were 101.27%, 99.72% and 98.86% for the partially purified avermectin treatment, Proclaim insecticide and uninfested control, respectively.

The protein content in fruiting bodies was significantly reduced to 18.51% in the insect-only treatment, while no significant differences were observed among the partial avermectin, Proclaim and uninfected control treatments, which recorded 35.21%, 34.78% and 34.59%, respectively.

Molecular identification using the mitochondrial cytochrome oxidase subunit I (COI) primer is a precise technique for insect species diagnosis. Previous research

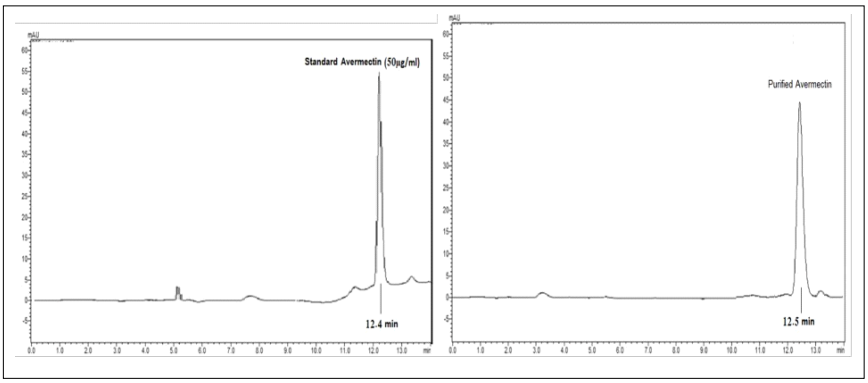


Fig 3: Standard curve from the analysis of Avermectin (right) and partially purified Avermectin from *S. avermitilis* strain Amna-4 (left) using HPLC.

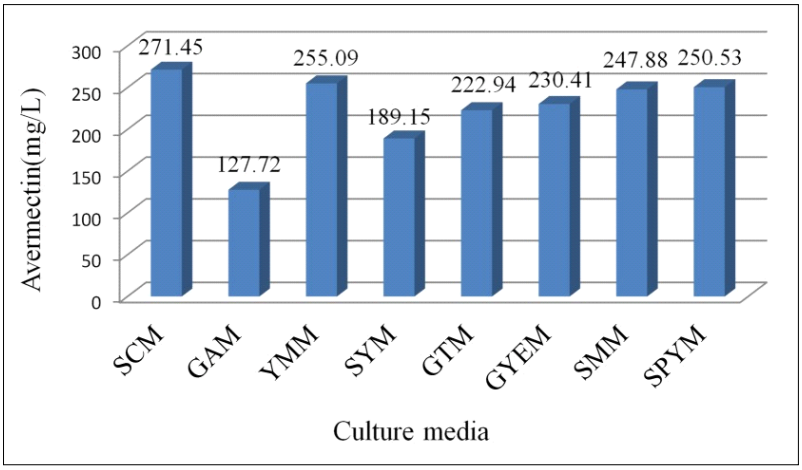


Fig 4: Effect of culture media on avermectin production from the intracellular extract of *S. avermitilis* (pH 8, incubation period, 12 days at 28°C), LSD (p≤0.05) = 4.11.

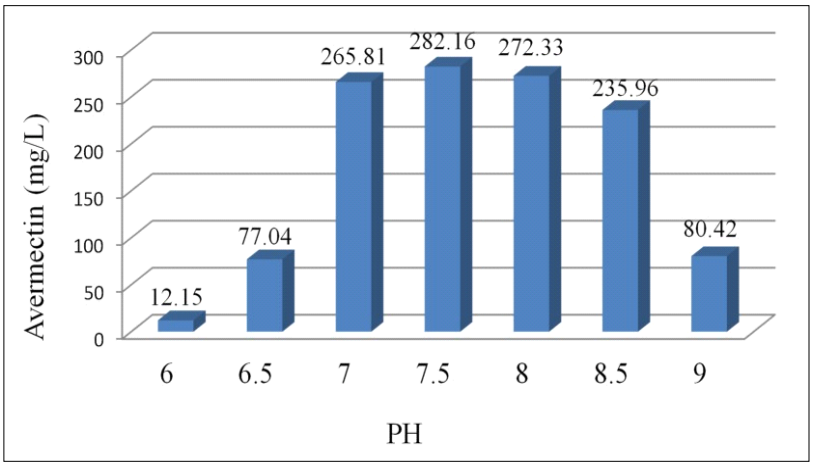


Fig 5: Effect of pH values on avermectin production from the intracellular extract of *S. avermitilis* (SCM medium, incubation period, 12 days at 28°C), LSD (p≤0.05) = 4.78.

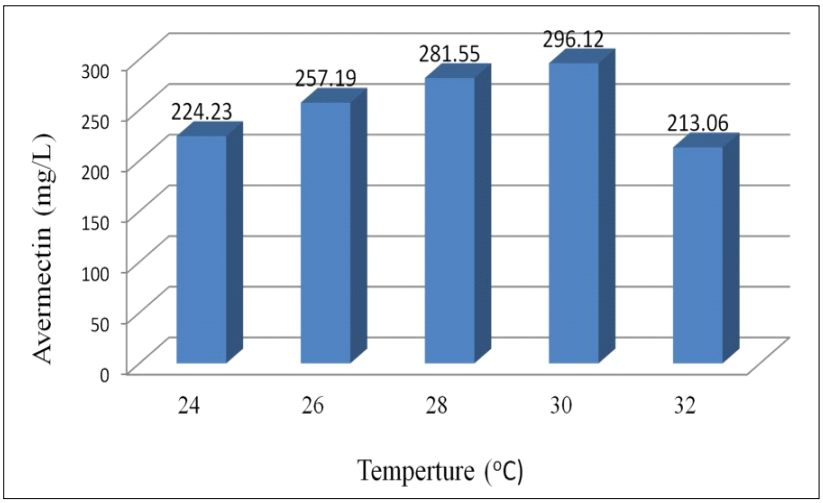


Fig 6: Effect of temperatures on avermectin production from the intracellular extract of *S. avermitilis* (SCM medium, pH 7.5, incubation period 12 days), LSD (p≤0.05) = 4.79.

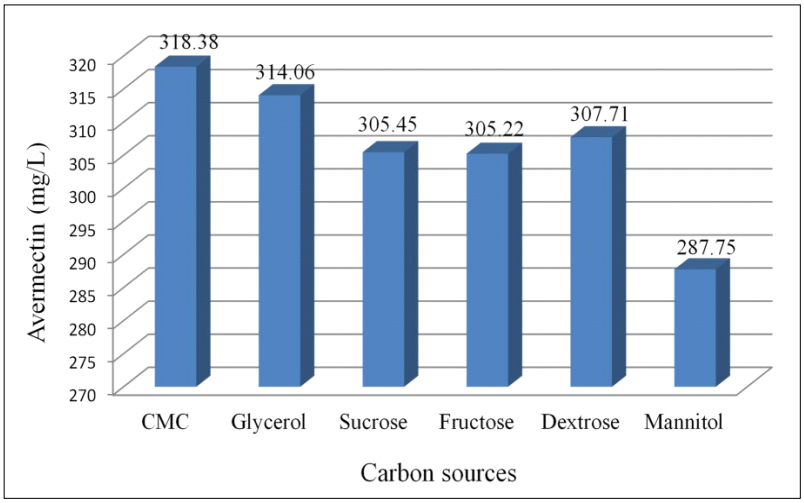


Fig 7: Effect of carbon sources at 5% on avermectin production from the intracellular extract of *S. avermitilis* (SCM medium, pH 7.5, incubation period 14 days, incubation temperature 30°C), LSD (p≤0.05) = 4.08.

has successfully used this gene to identify multiple insect species (Al-Shindah *et al*., 2022; Khalaf *et al*., 2024; Khalaf *et al.,* 2025). Results indicated that culture media play a significant role in the production of avermectin by *S. avermitilis*. Although all tested media were previously used for actinomycetes growth, variations in their compositions affect the production of secondary metabolites. High growth in a particular medium cannot be solely considered evidence of specific secondary metabolite production. The nutritional components are critical for activating secondary metabolism pathways in bacteria. Moreover, medium components influence the genetic regulation of metabolic pathways leading to avermectin biosynthesis. Liu *et al*. (2015) reported that culture media affect the expression of genes such as aveR, a key regulator of avermectin production. Other regulators such as AveT, a TetR-family transcription factor, positively regulate avermectin production by stimulating biomass factors and gene expression loci in the biosynthetic pathway. pH is an influential factor in avermectin production because proton concentration alters the efficiency of enzymes in the avermectin biosynthetic pathway and affects membrane permeability and ionization states of transporters involved in nutrient uptake. Slightly alkaline pH values (around 7-7.5) generally enhance the activity of peptidyl/polyketide enzymes responsible for chain elongation and macrocyclic ring formation. Thus, these pH values create a stable enzymatic environment for secondary metabolite production. Temperature also impacts cellular metabolic rates, enzyme stability and the morphological phase of *Streptomyces* spp. A mild increase in temperature up to 30°C may accelerate enzymatic reactions responsible for

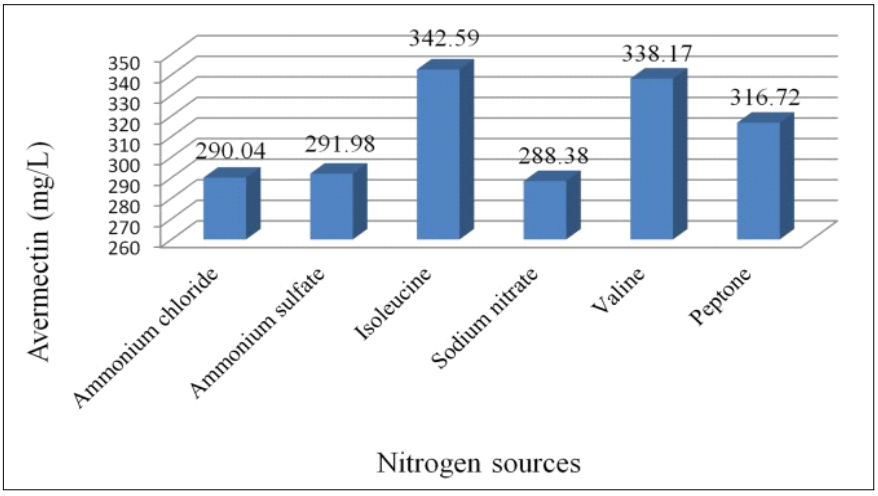


Fig 8: Effect of nitrogen sources at 5% on avermectin production from the intracellular extract of *S. avermitilis* (SCM medium, pH 7.5, with 5% of CMC, incubation period 14 days, incubation temperature 30°C), LSD ($p \leq 0.05$) = 4.44.

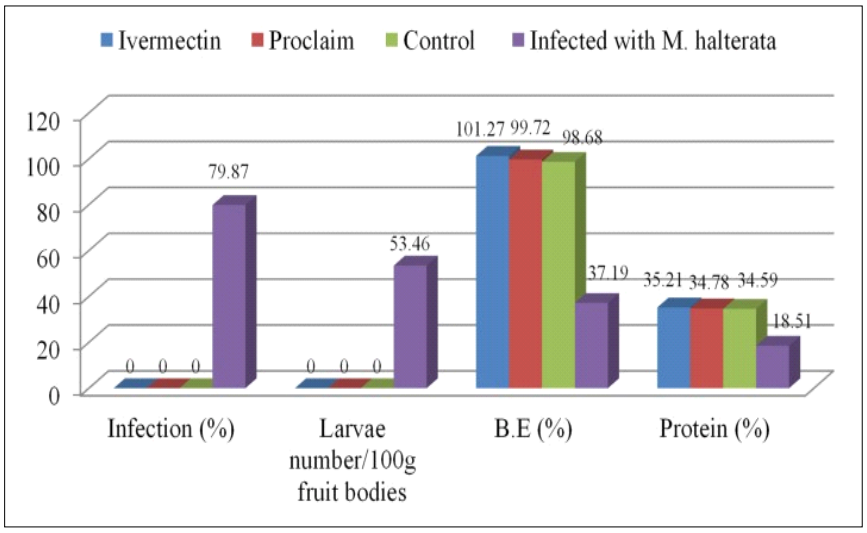


Fig 9: Effect of avermectin partially purified from *S. avermitilis* on the infestation rate of *M. halterata* compared to the commercial insecticide (Proclin) and the untreated control (without insect treatment).

polyketide chain elongation, but higher temperatures result in enzyme instability or heat stress, reducing production. Therefore, 30°C represents the balance between metabolic rate and pathway stability.

Secondary metabolite production in *Streptomyces* sp. typically follows "metabolic switching," where after the vegetative growth phase, the cell enters a distinct stage redirecting resources toward secondary metabolism. There is an optimal time point when cellular resources and enzymatic regulation balance to direct carbon flux toward avermectin biosynthesis; before this (vegetative growth), resources are insufficient and later, compounds may be consumed or subjected to regulatory repression. These findings correspond with the recognized temporal control of secondary metabolite production and align with Hassan and Mahmoud (2022) and Mahmoud and Hassan (2023), which highlighted the roles of temperature and incubation duration on the growth of various *Streptomyces* strains and their metabolites.

The types of carbon and nitrogen sources act as stimulatory or inhibitory factors for effective compound production, including avermectin. In the present study, among carbon sources, CMC was the most effective in increasing avermectin concentration, likely due to cellulose's role and bacterial cellulase production, which hydrolyzes it into simple glucose sugars easily assimilated by the bacteria. This role may be more physical than nutritional, as it helps modify culture morphology (pellet size/micro-aggregates) and alters oxygen diffusion and nutrient distribution within pellets, potentially enhancing secondary metabolite production in filamentous organisms. Well-distributed micro-morphology generally increases productivity. Ilić *et al*. (2008) demonstrated that CMC enhanced antibiotic production, including hexaene H-85 and azalomycine, by *Streptomyces hygroscopicus* CH-7. Their study also indicated that antibiotic production by filamentous organisms often depends on the form and size distribution of pellet populations in the culture medium. Adding polymer changed growth from a single large mass to reproducible, small pellets, minimizing wall growth.

Branched-chain amino acids (Ile and Val) are precursors for polyketide compounds (PKSs), which include many bioactive substances such as antibiotics, or for fatty acid chains related to the polyketides involved in the biosynthesis of avermectin (Batiha *et al*., 2020). They may also supply carbon/oxyl groups entering the avermectin biosynthetic pathways. Additionally, branched-chain amino acid degradation systems (*e.g*., branched-chain alpha-keto acid dehydrogenase; BCDH) regulate the provision of key units for avermectin biosynthesis pathways; thus, adding these amino acids can increase precursor flux toward avermectin and enhance production. Simple ammonium forms may cause repression of several secondary metabolite pathways (nitrogen catabolite repression), explaining reduced production with ammonium or nitrate salts. Furthermore, valine and isoleucine amino acids play a direct role in avermectin biosynthesis *via* the valine pathway producing isobutyryl-CoA and the isoleucine pathway producing α-methylbutyryl-CoA, both of which complete the biosynthetic route for avermectin (Batiha *et al*., 2020).

Partially purified avermectin treatment completely eliminated infections (zero infection rate), similar to the commercial pesticide proclaim, due to avermectin's efficacy as an insect neurotoxin that acts by long-term activation of glutamate chloride channels (GluCl) in nerves and muscles, leading to hyperpolarization, paralysis and insect death (Khan, 2023). Application of avermectin halts larval and adult nutrient uptake and mobility, preventing their feeding on *P. pulmonarius* fungus, thus preserving mushroom fruit bodies and maintaining protein content. The reduction in oyster mushroom yield and protein concentration caused by insects results from larval feeding on fungal tissues leading to tunnels and necrosis, which reduce fungal biomass and degrade fruit body quality, including protein content. The absence of infection due to partially purified avermectin treatment correspondingly preserved productivity and protein content.

CONCLUSION

The study successfully extracted and purified avermectin from *Streptomyces avermitilis* strain Amna-4, identifying the optimal production conditions. The insect *M. halterata* was molecularly confirmed as a parasite of *P. pulmonarius*. Partially purified avermectin showed similar chromato graphic properties to the standard and exhibited strong insecticidal activity, comparable to a commercial insecticide. It effectively prevented insect infestation without harming mushroom productivity or protein content.

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Disclaimers

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Informed consent

The manuscript does not contain animal experiments.

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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