

REVIEW

Of the *member* of the dissertation council for the dissertation of *Heakal Abdelhakem Abdelaziz Mohamed* on the topic: "Study of the antimicrobial properties of dispersive systems based on the Black Soldier fly (*Hermetia illucens*) larvae fat and the prospects of their use in medicine, veterinary, and crop protection", submitted for the degree of candidate of biological sciences in a scientific specialty 1.5.11 "microbiology"

Mr. Mohamed worked on Black Soldier fly (*Hermetia illucens*) "HI" based on larvae fat, which became in the last few years very attractive because of its high lipid and protein content, and natural antibacterial drugs with very high efficiencies, which could be developed. Although being AWME3 extract from HI completely isolated from the lipid part, it shows high activity against MDR microbes that are distributed in several fields, including agriculture, veterinary, and healthcare sectors. The development of new antibacterial agents from fat is a new technology, which will pave the way for solving many problems in these aspects. Since fatty acids in AWME3 play a crucial role, it is obvious that the research on these kinds of compounds with respect to activity and safety is prospective as well.

To optimize these materials in both directions of application a good understanding of the mechanism of action of fatty acids in AWME3 against MDR bacteria isolated from various sources is of high importance. Since sequential extraction with more FAs and glycerides in the dispersive system is a new technique to enhance the antimicrobial properties of FAs, it is obvious that also Mr. Mohamed developed this approach.

The structure of this dissertation includes an introduction, 7 chapters, a conclusion, a list of 391 references, and an appendix. It is written on 223 pages of typewritten text and includes 55 figures, 27 tables, and an appendix including 8 supplementary figures and 3 supplementary tables.

The introduction confirms and displays the relevance of the work, the aim of the study, the main objective, the formulation of unsolved problems, and the main tasks. Moreover, it indicates the scientific novelty, theoretical and practical significance of methodology and research of methods, statements submitted for the defense, degree of reliability and validation of results, grants which support the study, and dissertation structure.

Based on the thesis work results Mr. Mohamed published six articles among them, three articles were published in peer-reviewed journals indexed by Web of Science and Scopus (Frontiers in microbiology (IF=6.062), IJMS (IF=6.206), and Microorganisms (IF=4.95). Moreover, three articles were published in international conferences proceedings. Besides, the research findings were presented and discussed at 11 international conferences and webinars.

Chapter 1 "Literature review"

This part of the dissertation shows the problem under study and a brief theoretical summary of each chapter, which is divided into subtitles including the identification of *Hermetia illucens* (HI) fly and classification. Additionally, it shows the importance of HI larvae fat and blocks the gap between AMP and fatty acids as antimicrobials, where focuses on the larvae fat as a sustainable source of the antimicrobial agent; therefore it explains lipids classification, fatty acids classification, and the influence of rearing substrate on FAs content of BSFL. Furthermore, this chapter discusses in detail antibiotic resistance, mechanism of the antibiotic resistance, and the antimicrobial properties of SFAs and USFAs, which are abundant in HI larvae fat against MDR bacteria and biofilms, which cause many severe diseases in human, animal, and plants, and the mode of

action of these FAs and their applications in several fields such as agriculture, aquaculture, and health care sectors.

In brief, the literature review in Chapter 1 is extensive (about 241 citations), informative, and conclusive. It provides an excellent overview and motivation for this study.

Chapter 2 “Experimental methods of research”

This chapter illustrates the most developed methods and materials used to achieve the aim of the study. As far as I see, the descriptions are complete and should allow a reproduction of the material syntheses.

Chapter 3 “*Hermetia illucens* larvae fat eradicate phytopathogenic bacteria”

This chapter demonstrates the extraction of antimicrobial compounds of the HI larvae fat, identification, and testing of the isolated active compounds against five phytopathogenic bacteria, clearly showing that the extraction and bacterial eradication were successful. The main conclusion for this chapter is that the active compound biomass of AWME extract was 130 gm (4.33%). AWME effectively inhibited and eradicated the most important phytopathogenic bacteria.

Chapter 4 “Sequential extracts of *H. illucens* larvae fat eradicate pathogenic fish bacteria”

This chapter discusses the evaluation of the antimicrobial properties of three fractions isolated sequentially from the fat of HI larvae and nominated as AWME1, AWME2, and AWME3 that are tested against *A. hydrophila* and *A. salmonicida*. Furthermore, the identification of the chemical composition of each fraction was detected successfully using GC-MS. The total amount of bioactive compounds for each SE obtained was 60 mg, 40 mg, and 30 mg for AWME1, AWME2, and AWME3, respectively. The yield of consecutive SEs corresponds to 2%, 1.33%, and 1% for AWME1, AWME2, and AWME3, respectively, out of the initial 3 g of larvae fat. . However, the author should start with fractionation of the extracted material, but in my opinion, the argumentation in this chapter is very clear, and his findings are very fruitful for further optimizations.

Chapter 5 “Effect of *H. illucens* larvae fat AWME3 extract on XDR and MDR human pathogenic bacteria and eukaryotic HEK293 cells”

This chapter focuses on the eradication of hypervirulent *K. pneumoniae* strains: on the three hypervirulent *K. pneumoniae* strains: *K. pneumoniae* ATCC BAA-2473, *K. pneumoniae* KPM9, and *K. pneumoniae* KPi1627. The most interesting in this chapter is that AWME3 extract eliminated the human pathogenic bacteria strains but without toxicity to HEK-293 at the same concentration. Therefore, tested bacteria strains did not show any resistance to AWME3 extract, which opens the way for this novel drug to be more applicable against severe nosocomial bacterial infection and more sound in medical fields.

Chapter 6 “AWME3 from *H. illucens* larvae fat disrupt and eradicate biofilms formed by hypermucoviscus *K. pneumoniae* strains”

This chapter discusses the eradication of the planktonic and biofilms established by hypermucoviscus *K. pneumoniae* strains using AWME3 extract (Table 21). These findings enhance

the importance of AWME3 being an effective therapeutic drug applied in the healthcare sector, especially in intensive care units. In total, IZD, MIBC, MEBC, membrane permeability, and microscopical examination; clearly demonstrated the elimination of mature and immature biofilms (Fig. 33, 36, 37, and 38).

Chapter 7 “Study the mechanism of AWME3 action against MDR human pathogenic bacteria

The mechanism of action of AWME3 was studied on Gram-positive (*S. aureus* ATCC 55804) and Gram-negative (*A. baumannii* ATCC 19606) bacteria. It was clarified through several steps, including cell membrane permeability, rigidity, pore formation, cytoplasmic content leakage, electron transport chain, and nutrient uptake impairment. Afterward, cell wall degradation was followed by cell death; all of these were confirmed using several assays (Almar blue, bacteriolysis (killing time, growth curve kinetics), intracellular ATP, relative conductivity, cytoplasmic and genetic contents leakage at 260, 280 nm, respectively, and salt tolerance. Alterations in cell morphology, cell compartments, and cell dimension were visualized via microscopy techniques (SEM, TEM, AFM).

Finally, in the Conclusion, Mr. Mohamed summarizes the most important results in a numbered list and draws 13 conclusions from those (again in a numbered list). In my opinion, the discussion is well constructed and coherent, giving a good chance to better correlate results and conclusions to each other.

Dissertation of *Heakal Abdelhakem Abdelaziz Mohamed* on the topic: “Study of the antimicrobial properties of dispersive systems based on the Black Soldier fly (*Hermetia illucens*) larvae fat and the prospects of their use in medicine, veterinary, and crop protection " meets the basic requirements established by Order No.11181/1 dd. 19.11.2021 "On the procedure for awarding academic degrees at St. Petersburg State University". The applicant *Heakal Abdelhakem Abdelaziz Mohamed* deserves to be awarded the academic degree of candidate of biological sciences in a scientific specialty 1.5.11 “microbiology”. No violations of paragraphs 9 and 11 of the specified Order have been detected.

Member of the Dissertation Council

Signature

Date

Prof. Dr. Abdelazeem Mohamed Abdelazeem Algammal

A. Algammal

3/2/2023

Professor Doctor and Head of Department of Bacteriology,

Mycology, and Immunology.

Suez Canal University, Ismailia, Egypt.