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ANTIMICROBIAL ACTIVITY FOR EXCRETION AND SECRETION OF THE GREENBOTTLE FLY LARVAE *LUCILIA SERICATA* (MEIGEN) (DIPTERA: CALLIPHORIDAE)

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Abstract

Sterile larval excretion/secretion (ES) exhibited antibacterial activity against some species of bacteria. They were shown to inhibit the growth of Gram–positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* Gram–negative bacteria *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae* and Fungi *Geotricum candidum* and *Aspergillus fumigatus* thus exhibited limited inhibitory effect towards Gram–positive bacteria *Streptococcus pyogenes* and *Staphylococcus epidermidis* and Gram–negative *Proteous vulgaris* and Fungi *Syncephalastrum racemosum*, *Candida albicans*, that effect was slowed down when challenged with secretion on a solid media but no zone of complete inhibition was detected. Growth inhibiting activity was determined in liquid growth media using the Gram-positive, Gram–negative bacterial and fungal strains as indicator organisms.

Keywords: Antimicrobial activity, Antifungal activity, Maggot therapy, *Lucilia sericata*, Excretion/Secretion and Greenbottle fly larvae

Introduction

Effect of maggot, *Lucilia sericata* against biofilm of wound isolates *S. aureus* was carried out by Bohova *et al*. (2014), they suggested that maggot (ES) may act selectively against different bacterial strains. Most of excretion/secretion (ES) larvae of insects exhibit antibacterial activity against both Gram–positive, Gram–negative bacteria and fungi, especially *Lucilia sericata* (Valachova *et al*, 2013).

The maggots (ES) of *L. sericata* larvae (maggots) have effective activity for debridement and disruption of bacterial such as *Staphylococcus epidermidis*, The excretions/secretions (ES) activity was characterized according to concentration, incubation time and temperature, thermal stability, and size (Harris *et al*, 2013). Maggot's (ES) have a bactericidal and/or bacteriostatic activity against one or more of the bacterial species; the growth of Gram-positive bacteria is more reduced by maggots and/or their ES than the growth of Gram-negative bacteria (Cazander *et al*, 2009).

The present study aimed to the evaluation of *L. sericata* excretion and secretion on some pathogenic organisms as antimicrobial agent.

Materials and Methods

Insect used: The 3rd larval instar of *L. sericata* was collected from Tonamel Village, Aga Center, Al-Dakahia Governorate, Egypt and maintained at Medical Entomology Laboratory, Animal House, Zoology Department, Faculty of Science, Al-Azhar University, under controlled laboratory conditions of 27±2ºC and 70±10% RH and 12-12 light-dark photoperiod.

Collection of larval excretion/ secretion: Secretion and excretion of *L. sericata* maggots were collected as the following; washed sterile 3rd instar larvae (6000 larvae) with 70% ethanol and sterile ultrapure water (ddH_2O) and incubated overnight (10hrs) at 30°C, after incubation, centrifuged at 20,000×g for 15 min (kerridge *et al*, 2005).

Antimicrobial bioassay: Twelve pathogenic organisms were used for the antimicrobial assay; *Staphylococcus aureus*, *Bacillus subtilis*, *Staphylococcus epidermidis* and *Strep-*

tococcus pyogenes as Gram-positive bacterial strains, while the *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteous vulgaris* and the *Klebsiella pneumoniae* were used as Gram-negative bacterial strains. The fungi which used in this assay were *Aspergillus fumigatus*, *Syncephalastrum racemosum*, *Candida albicans* and *Geotricum candidum*.

The antimicrobial assay employed was broadly based on the standard agar diffusion assay at Regional Center for Mycology and Biotechnology Antimicrobial unit test organism, Al–Azhar University, Cairo, Egypt.

A colony of the tested microorganism was picked off a stock plate and suspended in Ringer's solution (10mL). An aliquot of the microorganism suspension (100mL) was swabbed onto agar plates (10mL agar). Excretion/ secretion products (25mL) were dispensed into the wells and the plates incubated at 35ºC for 24h. Radial zones of inhibition (mm) of bacterial growth around the sample wells were measured to determine the antibacterial activity. The diameter of growth-inhibition zone was standardized by inhibition zone of Ampicillin used as a positive control for Gram-positive bacterial strains, Gentamycin used as a positive control for Gram-negative bacterial strains and Amphotericin B used as a positive control for fungi occurrence (Holder and Boyce, 1994). The growth of inhibition zone was determined according Bulet *et al*., (1991).

Results

The antimicrobial activity of maggots ES against tested organisms:

Gram positive bacteria: The highest antibacterial (growth-inhibitory) activity of the maggots ES against Gram-positive bacterial was detected in *Staphylococcus aureus*, where the mean growth–inhibition zone was 18.3±1.2 mm vs. 27.4±1.5 mm for Ampicillin antibiotic, then followed by the *Bacillus subtilis*. The mean growth-inhibition zone was 22.6±0.58mm vs. 32.4±1.2 mm for the same antibiotic (Tab. 1 & fig. 1 A&B)

No antibacterial activity was detected in *Staphylococcus epidermidis* and *Streptococcus pyogenes* (Tab. 1 & Fig. 1 C&D).

Gram-negative bacteria:

The highest antibacterial growth–inhibitory activity against the bacterial strain Gram-negative was detected in *Escherichia coli*, where, the mean growth-inhibition zone was 20.4±1.0 mm vs. 22.3±0.72 mm for Gentamicin antibiotic, and then followed by the *Pseudomonas aeruginosa*, where, the mean growth–inhibition zone was 18.3±1.5mm vs. 22.6±1.5 mm for the same antibiotic (Tab. 2 & fig. 2 A&B)

No antibacterial activity was detected in *Proteous vulgaris* and *Klebsiella pneumonia* (Tab. 2 & Fig. 2 C&D)

Fungi: The most sensitive strain of tested fungi was detected in *Geotricum candidum*, where the mean growth-inhibition zone was 21.3±1.5mm vs. 23.2±2.1mm for Amphotericin B antibiotic, and then followed by the *Aspergillus fumigatus*, where as the mean growth-inhibition zone was 18.3±2.1mm vs. 22.6±1.5mm for the same antibiotic (Tab. 3 & Fig. 3 A&B)

No antifungal activity was detected in *Candida albicans* and *Syncephalastrum racemosumas* (Tab. 3 & Fig. 3 C&D).

Discussion

Lucilia sericata larvae possess antibacterial agents in their excretion/secretion, for this reason the antibacterial activity of these larvae was three times than that of *Sarcophaga carnaria.* These findings are in consistent with the observations noticed by Thomas *et al.* (1999), where they showed that, *Lucilia sericata* larvae were able to kill or decrease the total bacterial count of *Staphylococcus aureus* in vitro and to combat clinical infections in a variety of wound types including these caused by antibiotic resistance strains of bacteria, in agreement with the current study.

The results of the present study are comparable with those obtained by Jaklic *et al.*, (2008). They carried out in vitro and in vivo quantitative research to assess the effect of larval (ES) of *Lucilia sericata* on bacterial strains *Staphylococcus aureus*, most commonly colonizing the chronic wounds.

In agreement with the present study, Bexfield *et al.* (2004) showed that the excretion/secretion of *L. sericata* larvae has potent antibacterial activity against some pathogenic bacteria, including MRSA. Also, they concluded that the extract of the housefly, *Musca domestica* generally possess wide broad antibacterial activity against both Gram+ve and Gram-ve bacteria, this conclusion was in consistence with the present study.

The antibacterial properties of secretions aseptically collected from larvae of the greenbottle fly, *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) were examined by Kerridge *et al*. (2005) and they observed that there is antibacterial effective against Gram-positive *Staphylococcus aureus* and *Streptococcus pyogenes*, Gram-negative *Pseudomonas aeruginosa*, the present study is similar to the author in case of Gram-positive *Staphylococcus aureus* and *Streptococcus pyogenes* but, in consistent with Gram-negative *Pseudomonas aeruginosa*.

Daeschlein *et al*., (2007) found that the secretions of maggots, *L. sericata* are known to have antibacterial properties against *Micrococcus luteus*, *E. coli* and *S. aureus*, and this is similar with present study.

Generally, some of insect body extracts exhibit antibacterial activity against both Gram-positive and Gram-negative bacteria, for example, the silk worm, *Bombyx mori* (Hara and Yamakawa, 1995), the European bumblebee, *Bombus pascuorum* (Rees *et al*, 1997) their conclusion was in consistence with the present study. However, some insect species showed activity against only Gram-positive bacteria; for example; *Aedes aegypti* (Lowenberger *et al*, 1995) and *Chironomus plumosus* (Lauth *et al*, 1998).

Besides, Vizioli *et al.* (2001) reported that *Anopheles gambiae* and *Phormia terranovae* defensins displayed antibacterial activity spectra similar to that of other insect defensins as reported by Cociancich *et al.* (1994) against most of bacterial strains tested.

Killing of bacteria in the digestive tract of the maggot of *Lucilia sericata* (Diptera: Calliphoridae) was concluded by Mumcuoglu *et al*. (2001), indicated that the feces were either sterile or contained only small numbers of bacteria, this conclusion was in consistence with the present study.

In agreement with the present study Bexfield *et al*. (2007) demonstrated in vitro antibacterial activity of native excretion/ secretions (nES) from *L. sericata* against *B. cereus*, *E. coli* and *S. aureus*.

Evaluation of maggot excretions/ secretions are differentially effective against *S. aureus* and *P. aeruginosa* (Van der Plas *et al*, 2008), their results are similar with the present study.

Antifungal activity of maggots and their secretion: Hou *et al*. (2007) reported that the extract of the housefly larvae showed higher activity against Gram-positive bacteria than Gram-negative bacteria and did not show any antifungal activity and the present study disagreement with these results. In consistent with the present results,

The present study showed variable antifungal activity of excretion/secretion of *Lucilia sericata* against the filamentous fungus, *Asperigillus fumigatus*, the yeast, *Candida albicans*, *Synecephalastrum racemosum* and *Geotricum*. The different (ES) samples of *L. seictata* possessed the highest antifungal activities as compared with antibacterial activity, and possessed the highest antibacterial activities Gram-ve as compared with the Gram+ve, and also, showed that the bacterial strains tested were more sensitive to the dif-

ferent insect larvae used than the fungal strains tested. In agreement with these results, Meylaers *et al*. (2004) observed that, the last instar larvae of the housefly, *Musca domestica* displayed antifungal activity against, the *Saccharomyces cerevisiae* beside the antibacterial activity.

The present study declared the antibacterial activity against Gram+ve, Gram-ve bacteria and Fungi. In agreement with the present, Leem *et al*. (1999) showed that saw fly, *Acantholyda parki* extract was found to have a broad antibacterial spectrum against not only Gram-ve but also Gram+ve bacteria.

Conclusion

The outcome results showed that excretion and secretion of the green bottle fly larvae *Lucilia sericata* (Meigen) gave antimicrobial activities. Extensive work is ongoing and will published in due time.

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Table 1: Antibacterial activity as indicated by growth-inhibition zone of maggots (ES) of *L. sericata*, against Gram–positive bacteria.

Gram-positive Bacteria	Inhibition Zone/mm	Mean±SD Ampicillin (control)
Staphylococcus aureus	*18.3±1.2*	*27.4±1.5*
Bacillus subtilis	*22.6±0.58*	*32.4±1.2*
Staphylococcus epidermidis	-	*22.4±1.5*
Streptococcus pyogenes	-	*23.1±2.3*

SD: Standard deviation - : No inhibition zone

Table 2: Antibacterial activity as indicated by growth-inhibition zone of maggots (ES) of *L. sericata*, against Gram–negative bacteria.

Gram-negative bacteria	Inhibition Zone/mm	Mean±SD Gentamicin (control)
Pseudomonas aeruginosa	18.3±1.5	22.6±1.5
Escherichia coli	20.4±1.0	22.3±0.72
Proteous vulgaris	-	24.2±1.2
Klebsiella pneumonia	-	23.2±2.1

SD: Standard deviation - : No inhibition zone

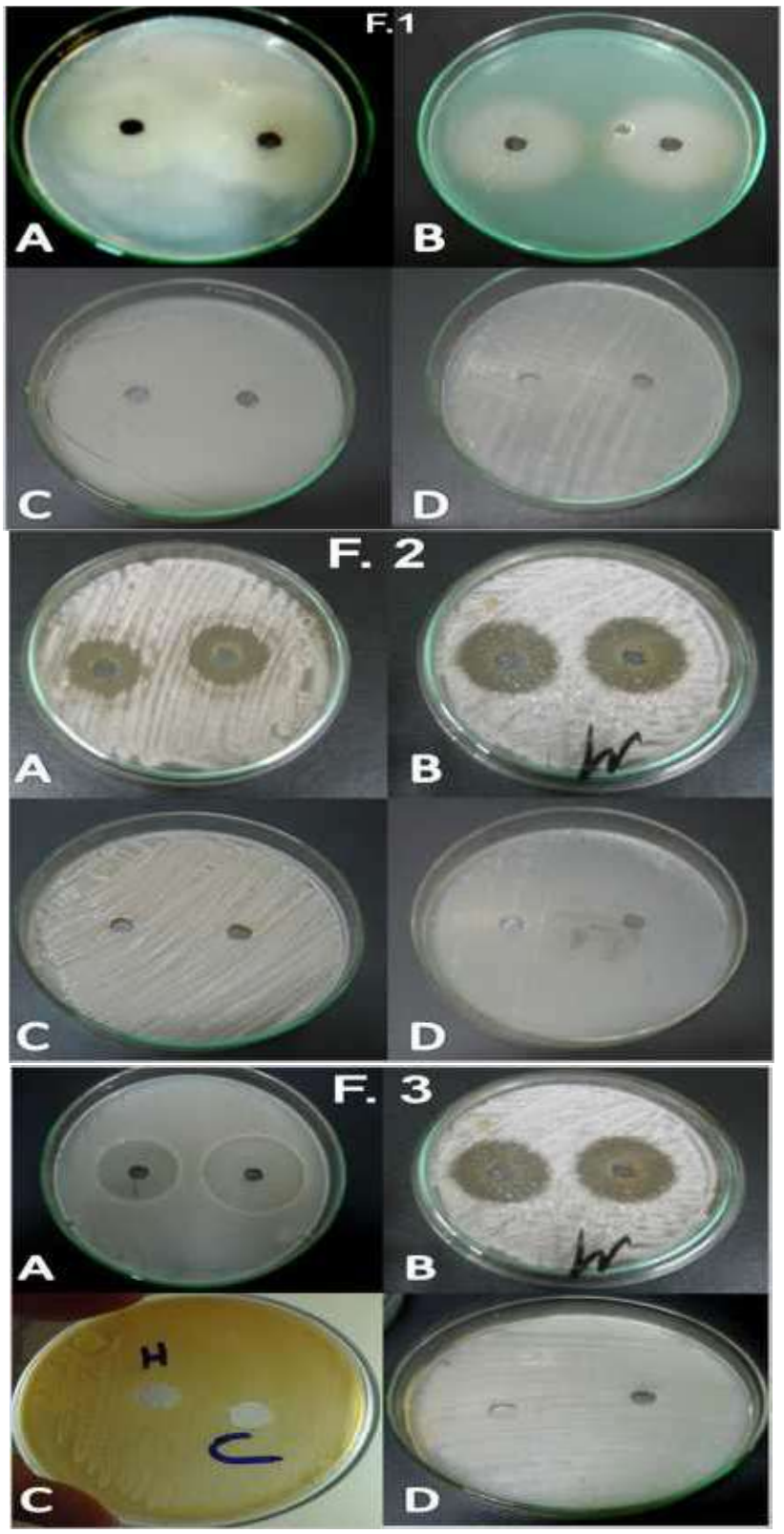
Table 3: Antifungal activity as indicated by growth-inhibition zone of ES of *L. sericata* maggots against strains of fungi.

Fungi	Inhibition Zone/mm	Mean±SD Amphotericin B (control)
Aspergillus fumigates	18.3±2.1	22.6±1.5
Geotricum candidum	21.3± 1.5	23.2±2.1
Candida albicans	-	24.2±1.2
Syncephalastrum racemosum	-	22.3±0.72

SD: Standard deviation
- : No inhibition zone

Explanation of figures

Fig. 1: Excretion and secretion effect of *L. sericata* larvae against Gram–positive bacteria (A) *Staphylococcus aureus*. (B) *Bacillus subtilis*. (C) *Staphylococcus epidermidi*. (D) *Streptococcus pyogenes*.
Fig. 2: Excretion and secretion effect of *L. sericata* larvae against Gram–negative bacteria (A) *Pseudomonas aeruginosa*. (B) *Escherichia coli*. (C) *Klebsiella pneumonia*. (D) *Proteous vulgaris*.
Fig. 3: Excretion and secretion effect of *L. sericata* larvae against fungal strains (A) *Geotricum candidum*. (B) *Aspergillus fumigates*. (C) *Candida albicans*. (D) *Syncephalastrum racemosum.*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