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Clinical and microbiological efficacy of medicinal maggots in the treatment of pressure ulcers in Egypt

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Abstract

Pressure ulcers are a significant public health problem, especially for patients in long term care facilities. The aim of this study was to assess the clinical and microbiological efficacy of maggot therapy in the treatment of pressure ulcers. The study was conducted on 14 bed-bound patients with 14 pressure ulcers at Alexandria Main University Hospital, Alexandria, Egypt. The blow fly *Lucilia sericata* was used for maggot therapy. Each ulcer was treated by one maggot cycle of 3 days per week. The ulcers were investigated weekly for changes in their size, the size of necrotic tissue, and the bacterial burden before and after each maggot cycle. Of the 14 ulcers treated with maggot therapy, three ulcers were completely debrided of which one ulcer needed only one cycle and two ulcers were debrided by two cycles. Nine ulcers had > 50% of their size occupied by a red healthy granulation tissue during a mean period of 2.14 weeks. The mean of initial bacterial burden of ulcers was significantly decreased from 4.86×10^8 CFU/ml exudate to 1.92×10^4 CFU/ml exudate (p=0.01814) below the 10^5 threshold of natural healing after the first maggot cycle. The clinical and microbiological outcomes demonstrate that maggot therapy is a rapid, simple, efficient, and cost-effective tool for treating pressure ulcers which do not respond to conventional treatment and surgical intervention.

Key words: maggot therapy, *Lucilia sericata*, pressure ulcers, Egypt

Introduction

Pressure ulcers, one type of chronic wound, remain a significant problem in both acute and community health settings (Bergstrom et al. 1994; Whitney et al. 2006; Spilsbury et al 2007; Tachibana et al. 2016). Pressure ulcers are not a plague of modern man but have been known to exist since ancient Egyptian times and have even been identified in the remains of mummified Egyptian priestess (Hampton 2005). The impact of pressure ulcers is significant in terms of both financial and non-monetary costs. Non-monetary costs, often described as the hidden costs of pressure ulcer care, include the emotional and physical impact on patients and their family caregivers. These patients reported a profound impact on many aspects of their lives (physical, social, financial), as well as loss of independence and control (Dharmarajan and Ugalino 2002; Niezgoda and Mendez-Eastman 2006; Spilsbury et al. 2007; Amir et al. 2017).

A pressure ulcer is defined as any lesion caused by unrelieved pressure resulting in damage to underlying tissue (Amir et al. 2016; Tachibana et al. 2016). The development of pressure ulcers is increased when the duration of immobility is increased. Once a pressure ulcer develops, this increases nursing care time, hospital care, infection, and mortality (Allman et al. 1995, 1999; Amir et al. 2017).

Despite the many developments in wound care, there has been no significant decrease in pressure ulcer prevalence or any demonstrable improvement in overall outcomes (Niezgoda and Mendez-Eastman 2006; Whitney et al. 2006; Amir et al 2017). New treatment paradigms must be examined as we strive to reduce pressure ulcer morbidity. For centuries, the infestation of wounds by certain species of fly larvae (maggots) has been recognized to debride, enhance healing, and decrease the mortality associated with underlying injury (Sherman et al. 2000).

The past three decades have witnessed the resurgence in the use of maggot therapy in wound management due to the recognized limits of conventional medical and surgical treatments, the increase of antibiotic resistance and the growing data demonstrating the safety and efficacy of this simple, low cost modality (Trudgian 2002; Thomas 2006; Sherman et al. 2013). Maggot therapy (also known as maggot debridement therapy, larval therapy, or biosurgery) is the medical use of live blow fly larvae to treat nonhealing infected wounds (Sherman et al. 2000). Maggots act in three primary ways; debriding (removal of necrotic tissue), disinfecting (killing bacteria), and promoting production of granulation tissue (Sherman 2014; Sherman et al. 2013).

Kotb et al. (2002) have conducted the first scientific study in Egypt on the use of medicinal maggots in the management of venous stasis and diabetic foot ulcers. Tantawi et al. (2007) have investigated the clinical and microbiological efficacy of maggot therapy for treating diabetic foot ulcers in Egypt. The outcomes of these studies have paved the way to investigate the utility of maggot therapy in the treatment of one of the most common type of chronic wounds, pressure ulcers.

The present study aims to investigate the clinical (debridement and wound healing) and microbiological outcomes of maggot therapy in the management of pressure ulcers.

Materials and Methods

Patient selection

Fourteen bed-bound adult patients with 14 sacral and ischial pressure ulcers of stage III and stage IV over a period of 14 months were included in the study at the general medical surgical wards, critical care units, and emergency recovery room at Alexandria Main University Hospital, Alexandria, Egypt. Permission to conduct the study was obtained from the ethical committee of the Faculty of Medicine, Alexandria University.

Clinical evaluation

Patient and wound histories were collected directly from the patients and their medical records. An initial assessment to the ulcers (site, age, and staging) was done according to NPUAP (2001). The circumferences of ulcers and necrotic tissue were determined by wound tracing and then measured by a mechanical planimeter. Ulcers were evaluated visually and photographically. Hemoglobin and albumin levels were measured.

Maggot therapy protocol

During this study, the blow fly *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) were reared and disinfected for medical use at the Maggot Therapy and Microbiology Laboratory, Faculty of Science, Alexandria University. The method of disinfection of *L. sericata* eggs is that of Kotb et al. (2002). The maggot therapy dressing used in our study is that of Kotb et al. (2002) and Tantawi et al. (2007) (Fig.1.) Each ulcer was treated with one maggot cycle of three days per week. At the end of each cycle, maggots were removed by pealing back the dressing with one hand while wiping up the larvae with a wet gauze pad held in the other hand and then destroyed by incineration. In between maggot cycles, patients regularly received sterile saline wet-to-dry gauze dressing. Maggot cycles were regularly applied until complete (100% of wound bed is free from devitalized tissue) or adequate (80-90% of wound bed is free from devitalized tissue)

debridement of the wound is attained within a period of 3 months.

Wound evaluation after maggot therapy

Changes in size of devitalized tissue and granulation tissue over time were recorded. Surface area and percentage of devitalized tissue were assessed each week. Degree of debridement was determined as the percentage of the surface area being debrided to the initial surface area of the wound. Rate of wound healing, if examined, was calculated as the average percent change (decrease) in the initial surface area of devitalized tissue of the wound per week. Wound color and odor and presence or absences of granulation tissue were recorded after each maggot cycle.

Microbiological procedures

During the present study, samples were collected from ulcers before and after each maggot cycle to investigate and to determine the bacterial load (burden), polybacterial population and the type of bacteria in each ulcer. Sterile cotton swabs were used to collect samples from the surface and subsurface of different representative locations in the ulcers. Fully exudates-saturated swabs (each loaded with ~ 250 µl) were taken from each ulcer and instantly immersed in LB-broth in 10-ml sterile screw-capped tubes as a transferring medium. Each tube contained sterile 2.5 ml broth. Bacterial cells were released from the swabs by vortexing for 3 min. to have a ten-time diluted bacterial suspension. This suspension represented a one tenth concentration of bacteria.

A serial dilution technique was used to count the Colony Forming Units (CFU) of bacteria in the previous suspension per each swab (250 µl). Obtained counts were multiplied by four to give the CFU/ml exudates which represent the loads of the bacteria per 1 ml exudates. From the dilution plates, colonies with different morphology and growth behavior isolated from different ulcers were picked up and purified by streaking on LB-agar plated before being subjected to preliminary and biochemical identification tests. Preliminary identification was done by the appearance of colonies on the agar plates.

The identification of bacteria was based upon feeding a database for the probabilistic identification program designed by Microsoft® (Bryant 2004). Gram's staining microscopic examination was done, as well as, catalase test, urease test, indole production, citrate utilization, lactose and glucose fermentation, nitrate reduction, hydrogen sulphide production and oxidase production, as key confirmatory and differentiating tests between wound bacterial flora. The used media have been prepared according to Atlas (1997).

Statistical analysis

Differences in surface area of necrotic tissue and granulation tissue of ulcers before

and after maggot therapy were statistically evaluated using Student's t-test (Sokal and Rohlf 1981).

Results

A group of 14 patients (10 males and 4 females) with 14 pressure ulcers was enrolled in this study. The mean age of patients was 43.35 years (range 18-81 years) (Table 1). All patients were with altered level of consciousness except only two patients were conscious. The patients were generally suffering from prolonged hospitalization with complete bed ridden with a mean duration of 11.28 weeks (range 0.7-49.1 weeks). The mean albumin level was 2.54 g/dl (range 2-3.1 g/dl) indicating that these patients were immunocompromised.

The characteristics of the 14 pressure ulcers before undergoing maggot therapy are shown in Table 2. Seven wounds were staged as stage III and the other 7 wounds were as stage IV. Ten ulcers were found on the sacrum, and 4 ulcers were on the right ischia. All the ulcers were previously treated with the conventional non-surgical methods available at Alexandria Main university Hospital; only six ulcers had undergone sharp debridement. The ulcers had a mean surface area of 90.7 cm^2 (range 28.6-154 cm^2). Necrotic tissue represented a mean of 61.73 % (range 18.5-

100%) of the size of ulcers. The mean age of ulcers was 9.78 weeks (range 2-15 weeks).

Maggot therapy was associated with a rapid rate of debridement (Table 3, Fig. 2). Before maggot therapy, the mean surface area of devitalized tissue was 58.81 cm^2 (range 8.25-131.25 cm^2), whereas after maggot therapy this mean significantly decreased to 15.35 cm^2 (range 0-40 cm^2) (p=0.000307) during a mean period of 1.5 weeks (range 1-2 weeks). This indicated that maggots decreased the size of necrotic tissue by an average of 43.46 cm^2. A mean of 27.68 % decrease in size of necrotic tissue was recorded per week. The mean number of maggot therapy cycles was 1.5 (range 1-2). Three ulcers (21.42%) were completely debrided by maggots of which one ulcer needed only one cycle and two ulcers were debrided by two cycles. Eleven patients had their ulcers not completely debrided because they died after undergoing one or two cycles of maggots. In nine ulcers (64.28%) more than 50% of the necrotic tissue was removed of which 5 ulcers were debrided with one cycle and four ulcers needed two cycles. In two ulcers (14.28%) less than 50 % of the devitalized tissue was removed with only one cycle.

Maggot therapy was also associated with rapid growth of granulation tissue (Table 4, Fig. 3). A red, healthy wound bed was

noticed in all cases after maggot therapy. Before maggot therapy, the mean surface area of granulation tissue was 16.03 cm^2 (range 0-80 cm^2), whereas after maggot therapy this mean significantly increased to 55.86 cm^2 (range 7.37-116 cm^2) (p=0.000221) during a mean period of 2.14 weeks (range 1-10 weeks). This indicated that maggots increased the size of granulation tissue by an average of 39.83 cm^2. Nine ulcers (64.28%) had > 50% of their size occupied by a red healthy granulation tissue. Figures 4 and 5 illustrate the clinical outcomes (debridement and granulation tissue growth) of maggot therapy in two pressure ulcers.

A total of 20 bacteria were isolated from the 14 ulcers examined. These bacteria were eight Gram-positive (7 aerobic and 1 anaerobic) and 12 Gram-negative (10 aerobic and 2 anaerobic) (Table 5). All ulcers exhibited a mixed bacterial population ranged from three to seven microorganisms (Table 6).

Maggot therapy was associated with marked antimicrobial activity (Table 7). The mean of initial bacterial burden was 4.86×10^8 CFU/ml exudate. After the first maggot cycle, this mean has significantly decreased to 1.92×10^4 CFU/ml exudate (p=0.01814) below the 10^5 threshold of natural healing.

The maximum fold of decrease in bacterial count was recorded in ulcer 6 (2.15×10 fold) and the minimum was observed in ulcer 12 (4.6×10^6 fold) (Table 7). The second maggot cycle was applied to six ulcers only because eight patients had died. The mean bacterial burden of the six ulcers before the second cycle was 5.61×10^5 CFU/ml exudate. This mean significantly decreased to 1.36×10^7 CFU/ml exudate after the second maggot cycle (p=0.1455). The maximum fold of decrease in bacterial count after second cycle was observed in ulcer 9 (4.29×10 fold) and the minimum was noticed in ulcer 12 (4.44×10^4 fold). It should be noted that the bacterial load of all ulcers was less than 10^5 CFU/ml exudate after the first or the second maggot therapy cycle.

Discussion

A significant development in the area of modern wound management is the recent re-introduction of maggot therapy for treating intractable, chronic wounds (Schultz et al. 2003; Kirshen et al. 2006). Maggot therapy has been successfully used in the treatment of pressure ulcers (Sherman et al. 1995; Sherman 2002), leg ulcers of venous, arterial, or mixed origins (Sherman et al. 1996; Thomas et al. 1996; Kotb et al. 2002; Davies et al. 2015), and diabetic foot ulcers (Mumcuoglu et al. 1998; Kotb et al. 2002; Sherman 2003; Gilead et al. 2012; Mirabzadeh et al. 2017).

During this work, maggot therapy was a rapid, simple, safe, well tolerated, and cost-effective tool for the treatment of pressure ulcers, which did not respond to conventional

treatment and surgical intervention. Maggot therapy was associated with rapid wound debridement. Complete debridement was achieved in 21.42% of the ulcers and significant debridement was achieved in 64.28 % of the ulcers of the patients with one or two maggot therapy cycles. Mumcuoglu et al. (1999), working on maggot therapy on patients with leg ulcers, pressure ulcers, and other types of wounds, found that the number of cycles needed to debride these wounds varied between one and 14 (mean, 6). In a maggot therapy study on diabetic ulcers, Mumcuoglu et al. (1998) stated that the number of cycles required for treatment varied between 1 and 23 (mean, 5.7). In the present study, 8.33% of patients have their ulcers completely debrided through one maggot therapy cycle and 14.28% of patients have their ulcers completely debrided through two maggot therapy cycles. Wayman et al. (2000) had obtained complete debridement in all six venous ulcers of patients enrolled in their study through only one maggot cycle. Kotb et al. (2002) have recorded complete debridement in 95% of the 30 venous leg ulcers belonging to 20 patients after treatment with one or two maggot cycles.

Tanyuksel et al. (2005) noticed that maggots completely debrided diabetic wounds varying in size from 6-84 cm^2 within 6-14 days through 1-6 cycles. Wolff and Hansson (2003) have investigated the effects of maggot therapy

on wounds in an open study of 74 patients with necrotic chronic ulcers of different etiologies. They found maggot therapy to effectively debride 86% of the ulcers to between 66 and 100%, and a single application or cycle was clinically beneficial in 72% of patients. They also noticed that no ulcer type was shown to be more suited to maggot therapy than others; however, ulcers in all 29 patients with diabetes were completely debride the venous ulcers of 16 of 20 patients with one cycle only. Also, in the same study, 6 of 11 diabetic patients had their ulcers completely debrided with only one maggot therapy cycle.

During this study, a mean of 28.97cm^2 of necrotic tissue per week was removed by maggots. This represented a mean of 27.68 % decrease in size of necrotic tissue per week. Sherman (2002) in his study on pressure ulcers, found that wounds treated with maggots showed a significant average decrease of 3-7 cm^2 of necrotic tissue within the first 2 weeks of therapy ($p< 0.001$). Also, in his study on diabetic foot ulcers, Sherman (2003) noticed that maggot therapy was associated with a significant decrease in necrotic tissue of a mean of 4.1 cm^2 within 2 weeks ($p= 0.02$), whereas conventional therapy was not associated with any decrease in necrotic tissue over the same period.

Tantawi et al. (2007) investigated the debridement efficacy of maggots on 13 diabetic

foot ulcers belonging to 10 patients. Complete debridement occurred in all ulcers in a mean of 1.9 weeks (range 1–4). The mean decrease in the percentage of necrotic tissue within the ulcer surface area was 64.7% (range 24.9–100) per week. Five ulcers required one cycle of maggot, five required two cycles, two required three cycles and only one ulcer needed four cycles to achieve complete debridement. After maggot therapy the mean ulcer surface area decreased significantly from $23.5cm^2$ to $2.3cm^2$ (p=0.001) in a mean of 8.1 weeks. This represented a mean 90% reduction in ulcer size, with a mean decrease of 16% per week.

Lucilia sericata larvae can only ingest liquid or semi-liquid food, since they have no teeth within the mouth (Sherman 2014). Necrophagous species feed on the dead tissue, cellular debris, and serous drainage of corpses or necrotic wounds (Zumpt 1965). Extracorporeal digestion by means of both amylase-containing saliva and proteolytic enzymes passed off in larval excreta is one mechanism by which wounds are cleansed (Sherman 2014; Nigam 2016). Debridement is also enhanced by the maggots' crawling about the wounds, probing and macerating it with their mouthhooks (Sherman 2014).

The results of this study have proved that maggot therapy is an effective method for initiating granulation. In a prospective controlled study to evaluate the utility of maggot therapy for treating pressure ulcers in spinal injury patients, it was found that wound healing was very rapid during maggot therapy than during antecedent conventional therapy (Sherman et al. 1995). Kotb et al. (2002) have investigated healing in 17 venous stasis ulcers belonging to 10 patients undergone maggot therapy. The mean surface area of these ulcers before maggot therapy was 57.70 cm^2, whereas after maggot therapy this mean decreased to 17.19 cm^2 (p< 0.05) during a mean period of 2.6 weeks (range 1-5.4 weeks). This indicated a mean of 70.21% decrease in size of ulcers. The authors also recorded that the ulcers healed at a mean rate of 32.98% per week. In two retrospective controlled studies to evaluate the utility of maggot therapy for treating pressure ulcers (Sherman 2002) and diabetic foot ulcers (Sherman 2003), maggot-treated wounds had faster rates of debridement and healing than conventionally treated wounds.

Although some early researchers believed that the larvae merely facilitated the normal healing process by eliminating necrosis and infection, it has now been shown that the larval digestive juices actually containing growth factors which stimulate healing (Sherman et al. 2013). Many clean wounds, requiring no debridement at all, heal faster when treated with maggots (Sherman 2002, 2014). The secretory/excretory products of maggots contain ammonia, urea and allantoin,

which are known to stimulate granulation (Sherman et al. 2000). In addition, maggot excretions contain calcium carbonate, which changes the wound pH from acid to neutral or slightly alkaline (pH 7 to 8), and which also has a stimulatory effect on granulation (Sherman et al. 2000).

Recently, it has shown that *Lucilia sericata* larvae secrete various cytokines and tissue growth factors (Sherman et al. 2013). With recent advances in cellular biology and chemistry, the secretory/excretory products of maggots stimulate the proliferation of fibroblasts and endothelial tissue, increases angiogenesis and oxygenation, and enhances fibroblast migration over model wound surfaces (Sherman 2014; Nigam 2016; Nigam and Morgan 2016).

In this study, 20 bacteria were isolated from the ulcers of which 60% were Gram-negative. Bowler et al. (2001) stated that pressure ulcers mainly harbor diverse and often dense microbial population involving both aerobic and anaerobic Gram-positive and Gram-negative bacteria especially *Staphylococcus* and *Entrobacter* species. Tantawi et al. (2007) has isolated 19 bacteria from 13 diabetic foot ulcers of which the Gram-positive were more than Gram-negative. It has been shown that *in vivo* (Steenvoorde and Jukema 2004) and *in vitro* (Thomas et al. 1999) trials, maggot therapy was more effective on

Gram-positive than Gram-negative bacteria. Steenvoorde and Jukema (2004) stated that reasons for faster growing of Gram-negative bacteria during maggot treatment could be attributed to the increase in pH of the medium exerted by maggots or that the endotoxins produced by Gram-negative bacteria are capable of destroying secretions produced by maggots. The authors concluded that a higher number of maggots is not only needed for a larger wound, or for a wound covered with a higher percentage of necrotic tissue, but also for Gram-negative infected wound.

Bacteriological examinations of infected wounds before initiating of maggot therapy, followed by subsequent cultures after maggot therapy, have shown that there is a marked decrease in infection (Wolff and Hansson 1999; Bowling et al. 2007). During this study, maggot therapy was associated with marked antimicrobial activity. One or two cycles of maggots were able to reduce the bacterial burden of each ulcer to less than the 10^5 threshold which facilitates natural healing (Schultz et al. 2003). Tantawi et al. (2007) have found that one cycle of maggots was able to decrease the bacterial load of all diabetic foot ulcers under investigation to less than 10^5 CFU/ml .

It has been now evident that medicinal maggots disinfect the wounds by three methods. First, the movement and irritation

caused by larvae ingesting liquefied necrotic tissue promote the formation of a serous exudate by the wound. This causes continuous wound lavage and dilution of bacterial concentration (Mumcuoglu 2001). Second, the larvae ingest bacteria that are then killed pass through their digestive tract (Mumcuoglu et al 2001). Third, the larvae secrete/excrete antibacterial compounds on the wound bed (Sherman 2014; Nigam 2016).

During this study, no complications related to maggot therapy were observed. All patients enrolled in this study accepted to undergo maggot therapy and did not complain of any major discomfort during the treatment. Patients were easily convinced to undergo maggot therapy by showing them or to their relative photographs of previous treatments or giving them a through explanation and appropriate literature about the advantages of this method. Only two patients (14.28%) complained of pain during treatment with maggots and were treated effectively with oral analgesics. It was also noted that the foul odour which was emanating from the ulcers of all patients has been dramatically reduced after maggot therapy. Similar findings and considerations were noted by Kotb et al. (2002).

Conclusions

The clinical outcomes (debridement and wound healing) demonstrate that maggot therapy is a rapid, simple, efficient, and cost-effective tool in the treatment of pressure ulcers which do not respond to conventional treatment and surgical intervention. The application of disinfected larvae of *Lucilia sericata* to infected, non-healing pressure ulcers of stage III and stage IV resulted in the rapid removal of necrotic tissue, disinfection, and enhancement of the healing process. All three actions are important in the preparation of the wound bed prior to healing.

Microbiological outcomes demonstrate that maggot therapy is an efficient antimicrobial treatment. Maggots were able to reduce the bacterial load of pressure ulcers below the 10^5 threshold which permits healing through one or two cycles.

Maggot therapy could alleviate the suffering in patients with pressure ulcers in Egypt. These patients represent a frail segment of the population and thus they are good maggot therapy candidates as surgical debridement is not an appropriate method for them. There is an overwhelming and urgent need for maggot therapy in developing countries, with poor medical facilities, to improve wound care.

Study limitation

As a result of increased mortality rate of the patients and deteriorations of their conditions this was led to the decrease in number of patients selected.

Table 1. Demographic and clinical characteristics of 14 patients with 14 pressure ulcers enrolled in maggot therapy

Age (years)	43.35 ± 4.18 (18-81)
Sex (n)	
Male	10
Female	4
Underlying medical conditions (n)	
Cerebral vascular stroke	11
Diabetes mellitus	6
Chronic obstructive pulmonary disease	9
Laboratory investigations	
Hemoglobin (g/dl)	
Male	09.68 ± 1.34 (8-13)
Female	08.67 ± 1.925 (6.5-11)
Albumin (g/dl)	02.54 ± 79.77 (2-3.1)
Duration of hospitalization (weeks)	11.28 ± 12.24 (0.7-49.1)

Data are means ± SD (range), unless otherwise specified.

Table 2. Characteristics of 14 pressure ulcers belonging to 14 patients before maggot therapy

Surface area (cm^2)	90.70 ± 41.38 (28.6-154)
Circumference (cm)	30.35 ± 07.34 (17-38)
Surface area of devitalized tissue (cm^2)	58.81 ± 42.33 (8.25-131.25)
% of devitalized tissue	61.73 ± 30.38 (18.5-100)
Age (weeks)	09.78 ± 04.07 (2-15)
Staging* (n)	
Stage III	7
Stage IV	7
Anatomical location (n)	
Sacrum	10
Rt ischial	4
Conventional treatment (n)	
Saline wet-to-dry gauze dressing, Betadine	14
Systemic antibiotics	14
Sharp debridement	6
Topical antimicrobial agents	10

Data are means ± SD (range), unless otherwise specified.

*Ulcers were staged according to NPUAP (2001).

Table 3. Clinical outcomes of debridement of 14 pressure ulcers belonging to 14 patients undergone maggot therapy

Initial surface area of devitalized tissue (cm^2)	58.81 ± 42.33 (8.25-131.25)
% of initial surface area of devitalized tissue	61.73 ± 30.38 (18.5-100)
Surface area of devitalized tissue (cm^2)	15.35 ± 13.21 (0-40)
% of surface area of devitalized tissue	20.21 ± 17.86 (0-69.8)
Duration until complete debridement (weeks)	1.5 ± 0.51 (1-2)
Decrease of surface area of devitalized tissue per week (cm^2/w)	28.97
% Decrease in surface area of devitalized tissue per week (%/w)	27.68
No. of maggot therapy cycles	1.5 ± 0.51(1-2)
No. (%) of ulcers completely debrided	3 (21.42)
No. (%) of ulcers with > 50% of debridement	9 (64.28)

Data are means $\pm$ SD (range), unless otherwise specified.

Table 4. Quality of wound bed of 14 pressure ulcers belonging to 14 patients undergone maggot therapy.

Initial surface area of granulation tissue (cm^2)	16.03 ± 22.97 (0-80)
Surface area of granulation tissue after maggot therapy (cm^2)	55.86 ± 33.90 (7.37-116)
Duration of therapy (weeks)	2.14 ± 02.41(1-10)
No. (%) of ulcers with >50% of their size covered by granulation tissue	9 (64.28)

Data are means $\pm$ SD (range), unless otherwise specified.

Table 5. Isolated bacteria from 14 pressure ulcers belonging to 14 patients

Gram-positive	Frequency of appearance
a. Aerobic	
• *Corynebacterium* sp.	3
• *Micrococcus* sp.	2
• *Staphylococcus aureus*	5
• *Staphylococcus* Coag. -ve	2
• *Streptococcus epidermidis*	6
• *Streptococcus pyogenes*	3
• *Enterococcus faecalis**	5
b. Anaerobic	
• *Peptostreptococcus sp.*	2
Gram-negative	
a. Aerobic & facultative	
• *Acinetobacter sp.*	2
• *Citrobacter sp.*	1
• *Enterobacter aerogenes*	3
• *Enterobacter cloacae*	4
• *Escherichia coli*	6
• *Klebsiella sp*	1
• *Proteus mirabilis*	5
• *Proteus vulgaris*	2
• *Pseudomonas aeruginosa*	4
• *Serratia liquefaciens*	1
b. Anaerobic	
• *Bacteroides sp.*	6
• *Propionibacterium sp.*	3

*(Previously Group D *Streptococcus*)
Coag +ve = coagulase positive, Coag –ve = coagulase negative.

Table 6. Number of bacterial strains isolates from each of 14 pressure ulcers belonging to 14 patients

Ulcer	Isolated bacteria	No. of isolated bacteria
1	*Staphylococcus aureus, Streptococcus epidermidis, Streptococcus pyogenes, Klebsiella* sp*., Pseudomonas aeruginosa, Bacteroides* sp.	6
2	*Streptococcus epidermidis. Enterobacter aerogenes, Proteus mirabilis, Pseudomonas aeruginosa, Serratia liquefaciens, Bacteroides* sp.	6
3	*Corynebacterium* sp., *Staphylococcus aureus, Streptococcus epidermidis, Bacteroides* sp, *Peptostreptococcus sp.*.	5
4	*Streptococcus pyogenes, Enterococcus faecalis, Enterobacter cloacae, Escherichia coli*	4
5	*Micrococcus* sp., *Streptococcus epidermidis, Proteus mirabilis, Propionibacterium* sp.	4
6	*Staphylococcus aureus, Staphylococcus* Coag. –ve, *Enterobacter aerogenes*	3
7	*Corynebacterium* sp., *Enterococcus faecalis, Enterobacter cloacae, Escherichia coli*.	4
8	*Corynebacterium* sp., *Streptococcus epidermidis, Acinetobacter* sp.	3
9	*Staphylococcus* Coag. –ve, *Streptococcus pyogenes, Escherichia coli, Proteus mirabilis, Bacteroides* sp, *Peptostreptococcus sp.*	6
10	*Staphylococcus aureus, Acinetobacter* sp., *Proteus vulgaris, Propionibacterium* sp.	4
11	*Micrococcus* sp., *Enterococcus faecalis, Enterobacter cloacae, Escherichia coli, Proteus mirabilis, Proteus vulgaris, Bacteroides* sp.	7
12	*Streptococcus epidermidis, Enterobacter aerogenes, Pseudomonas aeruginosa.*	3
13	*Staphylococcus aureus, Enterococcus faecalis, Enterobacter cloacae, Escherichia coli, Proteus mirabilis, Propionibacterium* sp.	6
14	*Enterococcus faecalis, Citrobacter* sp., *Escherichia coli, Pseudomonas aeruginosa, Bacteroides* sp.	5

Table 7. Bacterial burden of 14 pressure ulcers belonging to 14 patients before and after maggot therapy

Ulcer	Bacterial burden (CFU/ml)				Fold of decrease	
	Before MT1	After MT1	Before MT2	After MT2	MT1	MT2
1	7.40E+08	2.01E+04	-	-	3.68E+04	
2	9.20E+07	1.80E+04	-	-	5.11E+03	-
3	3.90E+08	5.50E+03	-	-	7.09E+04	-
4	1.20E+09	6.70E+02	-	-	1.79E+06	-
5	6.30E+07	7.40E+03	-	-	8.51E+03	-
6	7.10E+05	3.30E+04	-	-	**2.15E+01**	-
7	4.40E+07	8.50E+02	-	-	5.18E+04	-
8	8.40E+06	**4.20E+02**	-	-	2.00E+04	-
9	**6.80E+05**	2.80E+03	1.20E+05	2.80E+03	2.43E+02	**4.29E+01**
10	9.10E+06	8.10E+04	3.90E+06	**2.60E+04**	1.12E+02	1.50E+02
11	4.60E+08	7.20E+03	**4.80E+04**	**4.50E+02**	6.39E+04	1.07E+02
12	**2.90E+09**	6.30E+02	**7.10E+07**	1.60E+03	**4.60E+06**	**4.44E+04**
13	5.70E+08	4.80E+03	6.30E+06	2.20E+04	1.19E+05	2.86E+02
14	3.30E+08	**8.70E+04**	2.90E+05	5.20E+03	3.79E+03	5.58E+01
Means ± SD	4.86E+08 ± 779501673.9	1.92E+04 ± 29000.81364	5.61E+05 ± 28229993.42	1.36E+07 ± 11278.37533	4.84E+05 ± 1274542.528	7.50E+03 ± 18074.04166

Data are means ± SD (range), unless otherwise specified.
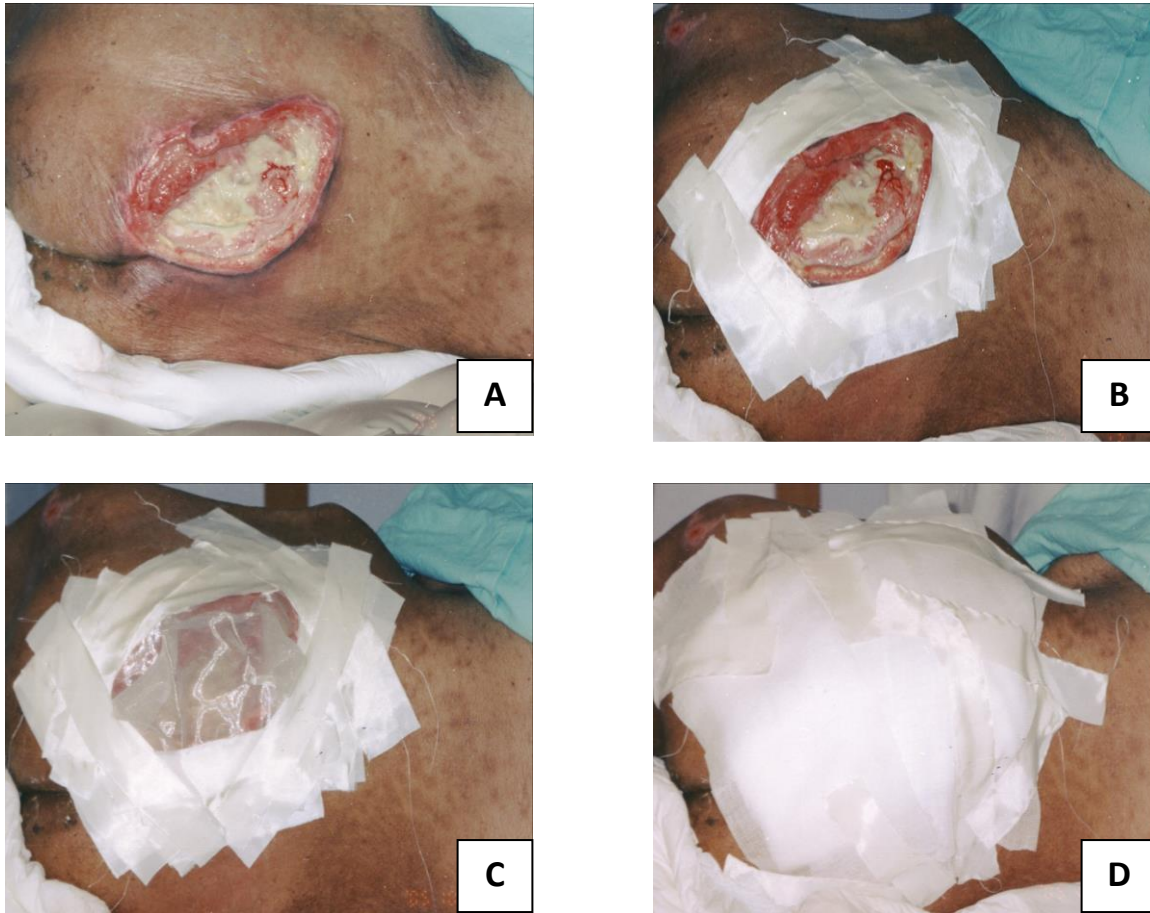MT1= first maggot therapy cycle.
MT2= second maggot therapy cycle.

Fig. 1. Method of application of maggot therapy dressing to the wound. A) Sloughy sacral ulcer; B) Adhesive silk plaster applied to the edges of pressure ulcer to protect the intact skin from the proteolytic enzymes secreted by maggots and to form the initial dressing; C) Nylon mesh applied to pressure ulcer to form a cage housing the maggots. Note the aggregation of young instars of *Lucilia sericata* beneath the mesh; D) Gauze sheet covering the mesh to strengthen the dressing, absorb wound exudate, and give an acceptable appearance of the treatment.
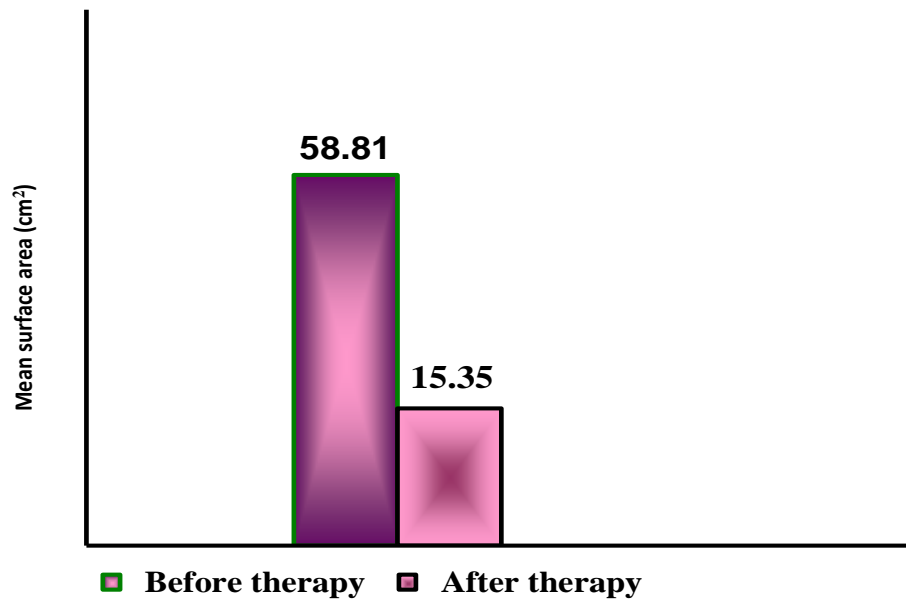
Fig. 2. Mean surface area of devitalized tissue of 14 pressure ulcers before and after maggot therapy.
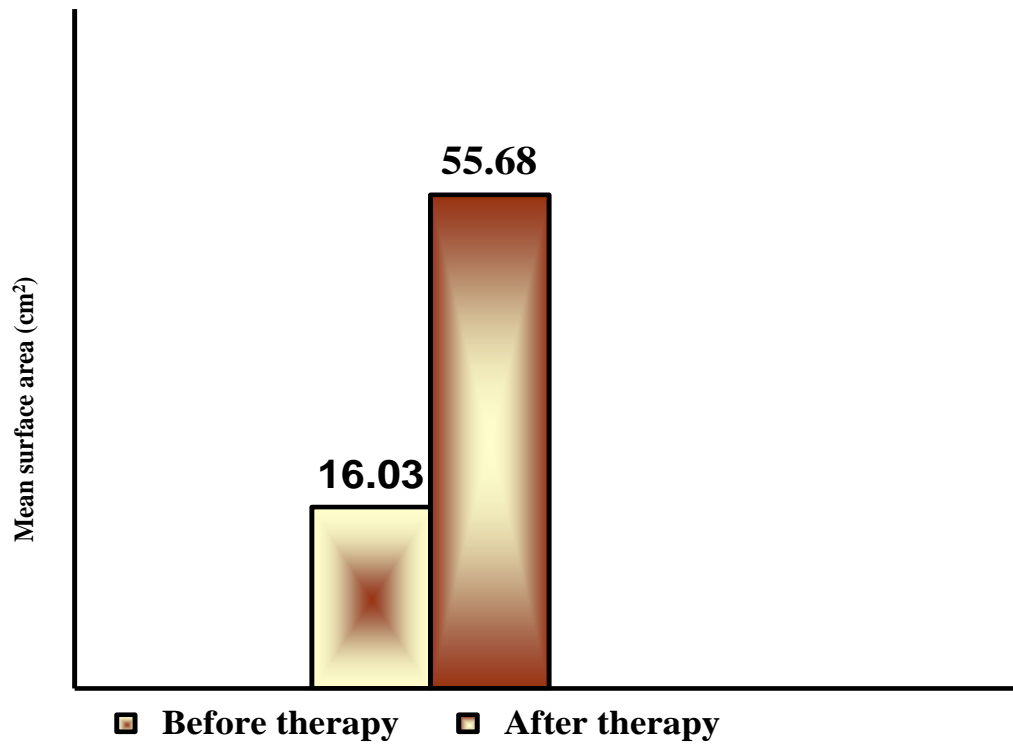
Fig. 3. Mean surface area of granulation tissue of 14 pressure ulcers before and after maggot therapy.

Fig. 4. Sacral pressure ulcer. A) before maggot therapy; B) after one week; and C) after 4   weeks of maggot therapy.

Fig. 5. Ischial pressure ulcer. A) before maggot therapy and B) after one week of maggot therapy.

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