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RELATIVE ABUNDANCE AND ANTIMICROBIAL ACTIVITIES OF BLOWFLY MAGGOT (*Lucilia robineau*) EXCRETION/SALIVA EXTRACT

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ABSTRACT

Maggots have long been used as a traditional way of cleansing and healing gangrenous wounds. Blowflies' abundance and antibacterial activities were determined using baited traps and agar cup plate technique. The family calliphoridae was the most abundant in all the sites sampled. The total of 5755 calliphorid flies captured belonged to two genera, *Lucinia robineau* (81.49%) and *Chrysomya megacephala* (18.51%). There was a significant difference in their abundance and a positive correlation between abundance and the two species with relative humidity and rainfall and a negative correlation with temperature. The isolates from infected wounds and whitlows were *Pseudomonas earoginosa, Staphylococus aureus, Klebsiella pneumoniae* and *Staphylococcus epidermidis.* Antibacterial susceptibility screening showed that, the maggot saliva/excretion was able to inhibit the growth. The zones of inhibition recorded was *Pseudomonas aeruginosa* was 24mm while Ampiclox (control) 37mm; *Staphylococcus aureus* was 20mm while Ampiclox (control) 31mm; *Klebsiella pneumoniae* was 27mm while Ampiclox (control) 39mm and *Staphyloccocus epidermidis* was 24mm while Ampiclox (control) 28mm. The result of the antibacterial susceptibility screening also revealed that, the greatest effect of the maggot saliva/excretion was obtained against *K. pneumoniae* while the lowest was obtained against *S. aureus*. Minimum Inhibitory Concentration (MIC) was between 40mg/ml and 60mg/ml while Minimum Bactericidal Concentration (MBC) screening showed that *Pseudomonas earoginosa, Staphylococus aureus, Klebsiella pneumoniae, Staphyloccocus epidermidis* have the same bactericidal concentrations of 60mg/ml each while Ampiclox (control) had 40mg/ml.

Keywords: Maggot, Saliva/excretion, Antibacterial, MIC and MBC.

INTRODUCTION

Blowflies are common insects found around public places such as the markets, abattoirs, refuse dumps, and even homes. Calliphoridae commonly known as blow flies, blowflies, carrion flies, blue bottles, green bottles, or cluster flies are a family of insects of the order Diptera, with 1,100 known species. The family is known to be polyphyletic, but much remains disputed regarding proper naming of the constituent units [1], some of which are occasionally accorded family status (e.g., Bengaliidae, Hselicoboscidae, Polleniidae and Rhiniidae).

Maggots of the blowfly have been successfully used as a debridement agent for chronic and infected wounds through history. First used by tribal people, maggot debridement therapy (MDT) was introduced into Western medicine following World War I by Baer [2] and extensively used to treat osteomyelitis and gas gangrenous wounds. Following the rediscovery of Alexander Fleming's penicillin from 1929 by Howard Florey and colleagues in 1939, the use of surgical maggots was abandoned [3]. However, due to the appearance of antibiotic resistance and increasing problems with chronic wounds worldwide, the treatment has seen a renaissance in modern medicine through the pioneering work by Church and Sherman [4] and other biotherapy advocates. The Food Drug Administration of the USA has approved MDT as a medical device, and maggots are produced aseptically and delivered by commercial companies to wound care centres and hospitals worldwide.

Maggots of the green bottle blowfly *L. sericata* are used for the treatment of many types of wounds including venous ulcers [5], traumatic and post-surgical wounds [6], osteomyelitis [7] and burns [8]. Maggots exert many effects that may be beneficial for wound healing. Earlier maggot excretions/secretions (ES) have been implicated in the breakdown of bacterial biofilms of *Staphylococcus aureus* and

Pseudomonas aeruginosa [9], [10]. Furthermore, maggots ingest and subsequently kill bacteria in their digestive tract [11], although quorum sensing regulated virulence factors from *P. aeruginosa* may be toxic to maggots [12].

The aim of this study is to determine the relative abundance and antibacterial activities of Blowfly Maggot (*Lucilia robineau*) Excretion/Saliva Extract.

MATERIALS AND METHODS

Study Area

Minna is the capital of Niger State, Nigeria. Minna experiences two distinct seasons (Dry and Wet season). The annual rainfall varies from about 1600mm in the south to 1200mm in the north of Minna Niger State. The duration of the rainy season ranges from 150 – 210cm from north to south. The minimum temperature of Minna fluctuates between March and June, while the maximum 33^0C to 30.6^0C is usually recorded between December and January. Most part of the state comes under the influence of the tropical continental air mass that blows from the north. Niger State lies between latitude 3.20^{o}' East and 9^{o}.25'N of the equator and between longitude 11.30^{o} North and 9^{o} 37' E of the equator. The population is 3,950,249. (National Population Census, 2006) (Figure 1).

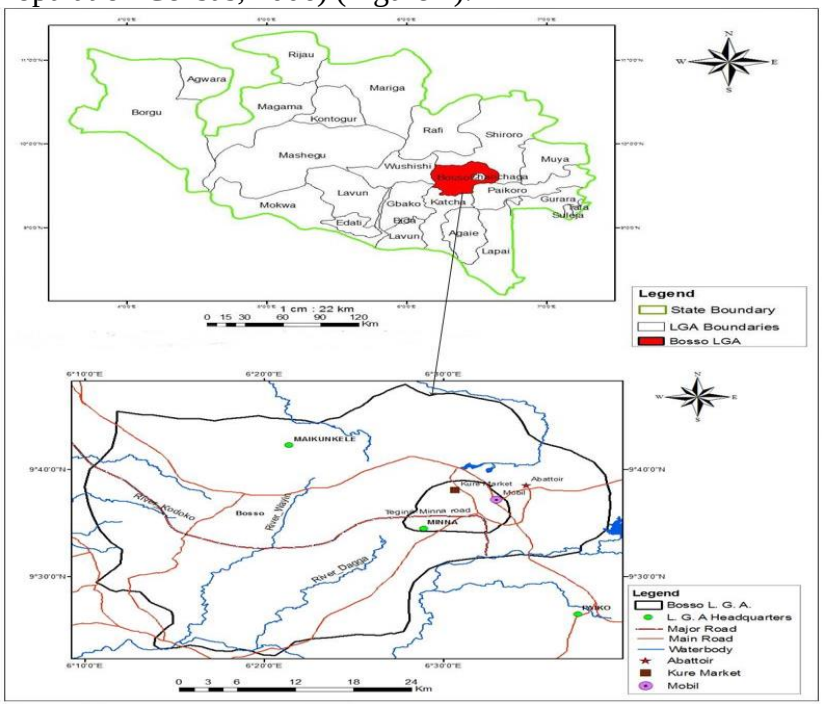

Figure 1: Maps of Niger State and Bosso LGA indication study areas (Abattoir, Kure market and Mobile pack).

Blowfly (Maggot) Sampling and Maintenance

Blowflies were sampled from three sites namely; Kure market, Mobile Park and Abattoir in Minna. At each site, four baited traps; each containing rotten meat was placed on the ground, at least 4 meters apart as described and modified by Ekanem [13]. Flies were collected at 2-hours intervals from 8.00 am to 2.00 pm local time.

Trapped flies were killed in the trap by dropping the cotton wool soaked in chloroform. Each site was sampled twice each month. Flies collected were counted, recorded, labeled and preserved

Raw meat were exposed to blowflies in Minna Abattoir for a period of one to two hours after which the eggs deposited in meat was placed in a nylon bag usually between 25-34ºC and kept till it decayed and maggots emerged. Maggots at the third instar pupated for adult emergence. These new adults were transferred to a fresh meat for egg laying and emergence of second generation maggots for the extraction of excretion/secretion of saliva.

Extraction of Maggot ES

The method described by Shuchi *et al.* [14] was used. Live adult specimens collected from the field were anaesthetized by chloroform for a short time for identification. After identification, the species were transferred to new cages for oviposition. Eggs laid on the meat were treated with 70% ethanol and sterile distilled water successively three times. The treated eggs were deposited on fresh meat and allowed to hatch to maggots for 2-3 days in an incubator at 35ºC. Late second or early third instar maggots were aseptically transferred to a flat petri-plate, washed with ethanol and sterile distilled water successively three times, and dried with filter paper. Treated larvae were incubated in sterile distilled water (5µl/larva) for 60 minutes at ambient temperature in the dark [15]. Resultant Maggot Extract (ME) obtained were transferred to another tube using a pipette and autoclaved for 20 minutes at 121ºC. Subsequently, the maggot extract is allowed to cool to room temperature and stored at -20ºC for analysis and future use.

***In vitro* studies to test for antibacterial activities and effects on antibiotic resistant wounds**

Swabs from active infected wound and whitlow were inoculated into 15ml sterile nutrient broth and incubated at 37^0c for 24hrs in accordance with the method described by Sharma [16]. These were later sub-cultured onto Manitol salt agar, Mac Conkey agar, Pseudomonas agar, Urea agar and Eosin methylene blue agar. The morphological characteristic of the isolates was determined using the gram staining technique as described by Sharma [16]. Biochemical tests were also conducted such as growing isolates on Catalase test, Coagulase test, Urease test and Oxidase test.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The Broth Dilution Method was used. The lowest dilution after incubation that showed no visible turbidity was regarded as the minimum inhibitory concentration and the concentration that showed no visible growth after incubation was considered the minimum bactericidal concentration (Rotimi and Mosadonmi, 1987) [17].

RESULTS

Three families of flies were identified namely; Calliphoridae, Muscidae and Sarcophagidae. In Kure market, the most abundant family was Calliphoridae 103539 (99.41%), Muscidae 316 (0.30%) and Sarcophagidae 300 (0.29%). In Mobile Park, the most abundant family was Calliphoridae 1832 (78.46%), Muscidae 380 (5.27%) and Sarcophagidae 123 (16.27%). While the family Calliphoridae was 1138.83 (65.01%), Muscidae 1093(12.33%) and Sarcophagidae 216 (22.58%) in Abattoir. There was a significant difference in the monthly abundance of flies in Minna. The most abundant family was Calliphoridae, followed by Muscidae and Sarcophagidae (Table 1).

Table 1: Abundance of flies captured in three locations from July 2014 – June 2015 in Minna.

Family/Sites	Kure market (%)	Mobile park (%)	Abatoir (%)
Calliphoridae	103539(99.41)	1832(78.46)	1138(65.01)
Sacophagidae	300(0.29)	123(5.27)	216(12.33)
Muscidae	316(0.30)	380(16.27)	397(22.58)
Total	104155	2335	1751

The total of 106509 calliphorid flies captured belonged to two genera, *Lucinia and Chrysomya*. Within the two genera, two species were identified: *Lucinia robineau* 35503(33.33%) and *Chrysomya megacephala* (Fabricius) 71006(66.67%) (Table 2). The female to male sex ratio of flies was 3:1, respectively. *Lucinia robineau* populations had five peaks in May, June, September, October and November while *Chrysomya megacephala* populations had five peaks in July, August, September, October and November. The two most common species were sampled in every month (Figure 2). *L. robineau* was most abundant in June while *C. megacephala* peaked in November.

Table 2: Abundance of Calliphoridae species captured in three locations from July 2014 – June 2015 in Minna.

Calliphoridae species	Female (%)	Male (%)	Total
Lucilia robineau	26628(75.00)	8875(25.00)	35503(33.33)
Chrysomya megacephala	53240(75.00)	17766(25.02)	71006(66.67)
Total	79868	26641	106509

Metrological data for temperature, rainfall and relative humidity were obtained from Geography Department, Federal University of Technology, Minna, Niger state. There was positive correlation between the two species of fly with relative humidity and rainfall and a negative correlation with temperature (Table 3).

Table 3: Pearson's correlation coefficients between numbers of species captured and meteorological data in three places from July 2014 to June 2015 in Minna.

Calliphoridae	Relative humidity	Temperature	Rainfall
L. robineau	0.815**	-0.461	0.591*
C. megecephala	0.401	-.670*	0.503

**= Correlation is significant at the 0.05 level.

**= Correlation is significant at the 0.01 level.

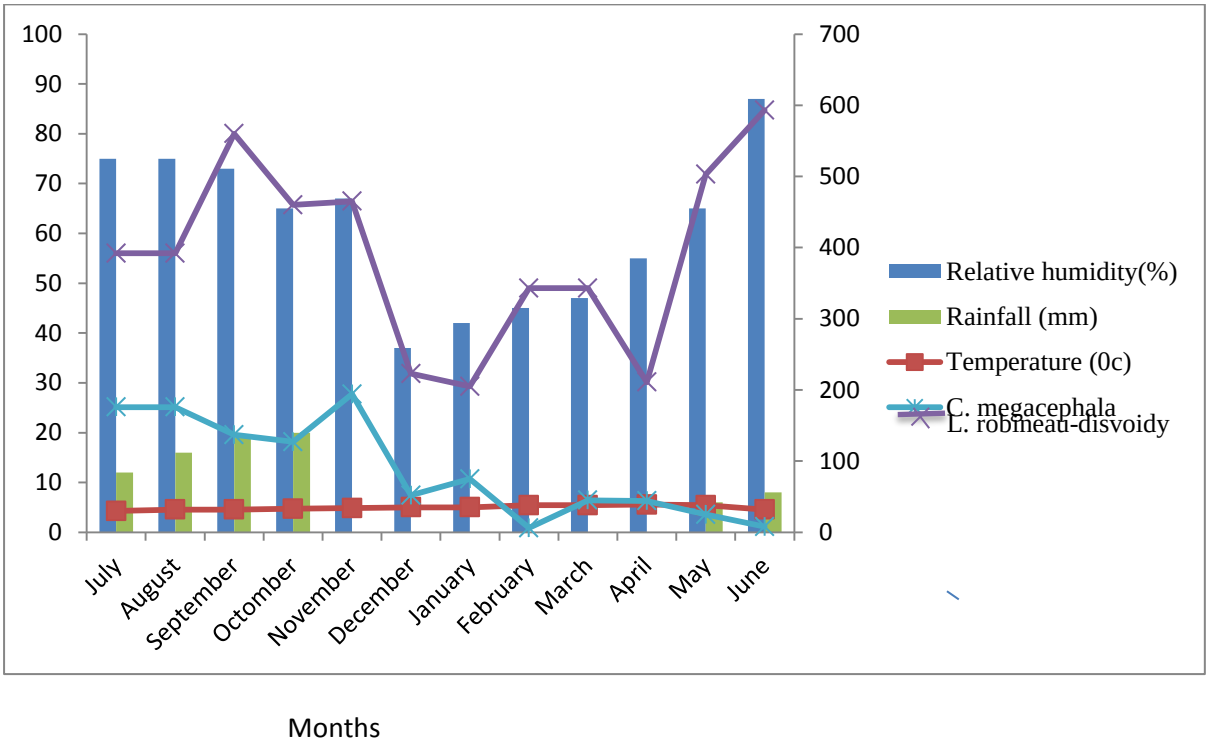

Figure 2: Relationship between climatic factors and number of species of calliphoridae.

The isolates from infected wounds were *Pseudomonas earoginosa, Staphylococus aureus* and *Klebsiella pneumoniae* while the one from whitlows was *Staphyloccocus epidermidis* (Gibson and Gordan, 1974) [18] (Table 4).

Table 4: Identification of the isolates from the infected wounds (including witlow)

Test	1	2	3	4
Gram reaction	_	_	+	+
Shape	Rod	Rod	Cocci	Cocci
Catalase	_	+	+	+
Coagulase	_	_	+	_
Urease	_	+	_	+
Oxidase	+	_	_	_
Organism identified	*P. earoginosa*	*K. pneumonia*	*S. aureus*	*S. epidermidis*

Antibacterial susceptibility screening of maggot excretion/saliva on infected wounds (including witlow)

Antibacterial susceptibility screening of Maggot metabolite of *Lucilia robineau* on test organisms showed that, after 24 hours incubation period, growth were observed on all the bacterial cultures, and the maggot extract was able to inhibit the growth. The zones of inhibition for *Pseudomonas aeruginosa* was 24mm while Ampiclox (control) 37 mm; *Staphylococcus aureus* was 20mm while Ampiclox (control) 31mm; *Klebsiella pneumoniae* was 27mm while Ampiclox (control) 39mm and *Staphyloccocus epidermidis* was 24mm while Ampiclox (control) 28mm (Table 5). The result of the antibacterial susceptibility screening also revealed that, the greatest effect of the maggot extract was obtained against K. *pneumoniae* while the lowest was obtained against *S. aureus*.

Table 5: Antibacterial susceptibility screening of maggots extract

Organisms	Mean Zones of inhibition (mm)	
	Maggot extract	Ampiclox (control)
Pseudomonas earoginosa	24	37
Staphylococus aureus	20	31
Klebsiella pneumonia	27	39
Staphyloccocus epidermidis	24	28

Minimum Inhibitory Concentration (MIC)

The results of Minimum Inhibitory Concentration (MIC) screening of the maggot ES was shown in Table 6. The result revealed that the MIC was between 40mg/ml and 60mg/ml. *P. earoginosa* was 60mg while Ampiclox(control) 40mg/ml; *S. aureus* was 50mg/ml while Ampiclox(control) 40mg/ml; *K. pneumoniae was* 60mg/ml while Ampiclox (control) 40mg/ml and *S. epidermidis* was 55mm while Ampiclox (control) 50mm. *P. aeruginosa* and *K. pneumoniae* has the highest inhibitory concentration while *S. aureus* has the least inhibitory concentration.

Table 6: Minimum Inhibitory Concentration (MIC) of maggots metabolite

Organisms	Maggot extract(mg/ml)	Ampiclox (control) (mg/ml)
Pseudomonas earoginosa	60	40
Staphylococus aureus	50	40
Klebsiella pneumonia	60	40
Staphyloccocus epidermidis	55	50

Minimum Bactericidal Concentration (MBC)

While the result of the Minimum Bactericidal Concentration (MBC) screening showed that *Pseudomonas earoginosa, Staphylococus aureus, Klebsiella pneumoniae, Staphyloccocus epidermidis* have the same bactericidal concentration. The result revealed; *P. earoginosa was* 60mg/ml while Ampiclox (control) 40mg/ml; *S. aureus* was 60mg/ml while Ampiclox (control) 40mg/ml; *K. pneumoniae* was 60mg/ml while Ampiclox (control) 40mg/ml and *S. epidermidis* was 55mm while Ampiclox (control) 50mm (Table 7).

Table 7: Minimum Bactericidal Concentration (MBC) of maggots metabolite

Organisms	Maggot extract(mg/ml)	Ampiclox (control) (mg/ml)
Pseudomonas earoginosa	60	40
Staphylococus aureus	60	40
Klebsiella pneumonia	60	40
Staphyloccocus epidermidis	60	40

DISCUSSION

The abundance of calliphorid species differed among the three areas of study, but individuals of the family Calliphoridae *Chrysomya megacephala* were much more abundant than *Lucilia robineau.* There is dearth of knowledge on the abundance of calliphorids in Nigeria. The observed patterns of abundance and species distribution are same from the results of calliphorid surveys from other countries like in urban Bangkok [18] where *Chrysomya megacephala* was the most abundant calliphorid, followed by *C. rufifacies*. Similar observations were reported in Brazilian localities such as Campinas, Rio de Janeiro, Curitiba, and some municipalities in the state of Goias [19]; where individuals of the family Calliphoridae were much more abundant than the other families in all three municipalities. *Chrysomya megacephala* was the most abundant species in the three municipalities, followed by *C. albiceps, C. putoria*, and *L. eximia*. It is not known why *Chrysomya megacephala* seems to be more abundant from these independent studies from different countries though they all have tropical climates in common, which would have favored this species.

Abundance correlated positively with relative humidity, rainfall, and a negative correlation with temperature. A similarly correlation between climate factors and the

abundance of blowflies was also observed in Malaysia, where the number of specimens were not associated with weather conditions at the time of trapping, but was positively correlated with the total rainfall [20]. Again, weather conditions from these areas are same which these species are more adapted to.

There are several reports on the antimicrobial activities of maggots [21], [22], [23]. Most studies have shown that maggots can excrete a complex mixture of substances into a wound that can clean the wound and stimulate its healing. Since multiple factors are responsible for the antibacterial activity of maggot excretions, it is evident that the efficiency of the excreta/secreta, and hence of larval therapy, depends on one hand on the 'quality' of the maggots, and on the other hand on the microbiological and physico-chemical conditions present in their immediate environment. Research towards alternative modes of antimicrobials is because of emergence of bacterial resistance to antibiotics.

Most studies on maggots are mainly on the species *Lucilia sericata*, however, in the present study the maggot of *Lucilia robineau* was identified to have antimicrobial properties. The excreta/secreta were able to inhibit the growth of *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae* and *Staphyloccocus epidermidis* isolated from wounds. This agrees with the work of Jaklič *et al.* [24] that studied the selective antimicrobial activity of maggot *Lucilia sericata* against pathogenic bacteria. They observed that maggot extract acted against Gram-positive bacteria, like *Staphylococcus aureus*, but less with Gram-negative bacteria, especially *Proteus* spp. and *Pseudomonas* spp. strains isolated from wounds. Also *in vitro* bactericidal activities have been reported for larval excretions of different maggot species for individual species, including *S. aureus, Streptococcus pyogenes*, *Enterococcus faecalis*, '*Clostridium welchii*', *Proteus vulgaris*, *S. pneumoniae* and *E. coli* [25], [26], [22], [23], [27]). Infected wounds that harbour different bacterial communities represent a varied, natural environment for maggots, which changes after their application in a relatively consistent and repetitive pattern. Both Minimal inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC) give some idea of the effectiveness of a chemotherapeutic agent against a microorganism. These indicate that the excreta/secreta of the maggot of *Lucilia robineau* has antibacterial activity.

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REFERENCE

1. Yeates, D. K. & Wiegmann, B. M. (1999). "Congruence and controversy: toward a higher-level phylogeny of Diptera" (PDF). *Annual Review of Entomology*, 44, 397–428.

2. **Baer, W. S. (***1931***).** *The treatment of chronic osteomyelitis with the maggot (larva of the blowfly). J Bone Joint Surg Am* 13, *438–475.*Howard Florey and colleagues in 1939

3. **Pechter, E. A. & Sherman, R. A. (***1983***).** *Maggot therapy: the surgical metamorphosis. Plast Reconstr Surg* 72, *567–570.*

4. Dumville, J. C., Worthy, G., Bland, J. M., Cullum, N., Dowson, C., Iglesias, C., Mitchell, J. L., Nelson, E. A., Soares, M. O, Torgerson, D. J. (2009). Larval therapy for leg ulcers (VenUS II): randomised controlled trial. *BioMedical Journal*, 338, b773.

5. Mumcuoglu, K. Y., Ingber, A., Gilead, L., Stessman, J. & Friedmann, R. (1998). Maggot therapy for the treatment of diabetic foot ulcers. Diabetes Care 21, 2030–2031.

6. Jukema, G. N., Menon, A. G., Bernards, A. T., Steenvoorde, P., Rastegar, A. T. (2002). Amputation-sparing treatment by nature: "Surgical" maggots revisited. *Clinical Infectious Disease.* 35, 1566–1571.

7. Sherman, R. A. & Pechter, E. A. (1988). Maggot therapy: a review of the therapeutic applications of fly larvae in human medicine, especially for treating osteomyelitis. *Medical Veterinary Entomology*, 2, 225–230.

8. Namias, N., Varela, J. E., Varas, R. P., Quintana, O. & Ward, C. G. (2000). Biodebridement: a case report of maggot therapy for limb salvage after fourth-degree burns. *Journal of Burn Care Rehabilitation,* 21, 254–257.

9. Van der Plas, M. J., Jukema, G. N., Wai, S. W., Dogterom-Ballering, H. C. & Lagendijk, E. L. (2008). Maggot excretions/secretions are differentially effective against biofilms of *Staphylococcus aureus* and *Pseudomonas aeruginosa. Journal of Antimicrobial Chemotherapy*, 61, 117–122.

10. Van der Plas, M. J., Dambrot, C., Dogterom-Ballering, H. C., Kruithof, S. & van Dissel, J. T. (2010). Combinations of maggot excretions/secretions and antibiotics are effective against *Staphylococcus aureus* biofilms and the bacteria derived therefrom. *Journal of Antimicrobial Chemotherapy*, 65, 917–923.

11. Robinson, W. & Norwood, V. H**. (***1933***).** *The role of surgical maggots in the disinfection of osteomyelitis and other infected wounds. J Bone Joint Surg Am* 15, *409–412.*

12. Andersen, A. S., Sandvang, D., Schnorr, K. M., Kruse, T., Neve, S., Joergensen, B., Karlsmark, T., Krogfelt, K. A. (2010). A novel approach to the antimicrobial activity of maggot debridement therapy. Journal of Antimicrobial Chemotherapy, 65, 1646-1654.

13. Ekanem, M. S., Idiong, M. O. and Usua, E. J. (2013). Synanthropic indices and baits preference of common non-biting flies (Dipter: Cyclorrhapha) of Akwa Ibom State, Nigeria. *International Journal of Biodiversity and Conservation*, 5(4): 192 – 197.

14. Shuchi A, Carl B, Chusing L. Maggot metabolite and combinatory effect with antibiotic *Staphlococcus aureus. Ann. Clin. Microbiol. Antimicrobes*. 2011;10:6.

15. Mariena JA, Cheryl D, Helen CM, Simone K, Jaap T, Peter HN. Combinations of maggot excretions/ secretions and antibioticsare effective against Staphlococcus aureus biofilms and bacteria derived fromrhereform. J. Antimicrobialchem. 2010;65(5):917-923.

16. Sharma (2007) Sherman RA. Maggot versus conservative debridement therapy for the treatment of pressure ulcers. *Wound Repair Regeneration*. 2002;10:208-214

17. Rotimi, V. O. and Mosadonmi, H. A. (1987). The effects of crude extract of nine African chewing sticks on oral anaerobes. *Journal of Medical Microbiology*, 23: 56 60.

18. Sukontason, K., Sukontason, K. L., Piangjai, S., Tippanun, J., Lertthamnongtham, S., Vogtsberger, R. C. & Olson, J. K. (2003). Survey of forensically-relevant fly species in Chiang Mai, northern Thailand. *Journal of Vector Ecology*. 28, 135–138.

19. Souza, A. M. & Linhares, A.X. 1997. Diptera and Coletoptera of potential forensic importance in Southeastern Brazil: relative abundance and seasonality. *Medical and Veterinary Entomology*, 11: 8 - 12.

20. Mahon, R.J.; Ahmad, H.; Wardhaugh, K. G. 2004. Factors affecting abundance and oviposition rates of a field population of the Old World screw - worm fly, Chrysomya bezziana (Diptera: Calliphoridae). *Bulletin of Entomological Research* 94: 359 - 368.

21. Grassberger, M. & Fleischmann, W**. (**2002**).** *The Biobag – a new device for the application of medicinal maggots. Dermatology* 204, 306

22. Pavillard, E. R. & Wright, E. A. **(**1957**).** *An antibiotic from maggots. Nature* 180, 916*–917.*

23. Simmons, S. W. (1935). A bactericidal principle in excretions of surgical maggots which destroys important aetiological agents of pyogenic infections. *J Bacteriol* 30, 253–267.
24. Jaklic, D., Lapanje, A., Zupancicia, K., Smrke, D., Gunde-Cimerman, N. (2008). Selective antimicrobial activity of maggots against pathogenic bacteria. *Journal of Medical Microbiology*, 57, 617-625.

25. Kerridge, A., Lappin-Scott, H. & Stevens, J. R. **(**2005**).** *Antibacterial properties of larval secretions of the blowfly, Lucilia sericata. Med Vet Entomol* 19, 333*–337.*

26. Lerch, K., Linde, H.-J., Lehn, N. & Grifka, J. **(**2003**).** *Bacteria ingestion by blowfly larvae: an in vitro study. Dermatology* 207, 362*–366.*

27. Wolff, H. & Hansson, C. **(**1999**).** *Larval therapy for a leg ulcer with methicillin-resistant Staphylococcus aureus. Acta Derm Venereol* 79, *320–321.*