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ASSESSING THE *IN VITRO* ANTIBACTERIAL EFFICACY OF *OCIMUM GRATISSIMUM* LEAF EXTRACT ON BOAR SEMEN

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ABSTRACT

Prolonged application of synthetic antibiotics promotes emergence of antibiotic-resistant bacteria. Thus, botanicals such as scent leaf rich in antimicrobial properties are being explored. Hence, effect of *Ocimum gratissimum* Leaf Extract (OgLE) on spermatozoa viability and fertilising potential in extended porcine semen was investigated in this study. Semen collected from a three-year-old Large White boar (250 kg) for isolation and identification of bacteria. Collected semen was extended at a ratio of 1:4 with Beltsville Thawing Solution (BTS) containing streptomycin and penicillin (T1), BTS without streptomycin and penicillin (T2), and different OgLE inclusions at 0.5% (T3), 1.0% (T4), 1.5% (T5) and 2.0% (T6) in BTS. The treatments were assessed *in vitro* at 0, 24, 48 and 72 hours at 17°C for Bacterial Load (Log_{10} CFU/mL) using the pour-plate method. Progressive Motility (PM) and acrosome Integrity, were assessed. Bacteria species identified were *Salmonella* species and *Escherichia coli*, with frequency of occurrence of 20.2% and 30.1%, respectively. Bacterial load in extended semen ranged from Log_{10} 0.13 (T3) to Log_{10} 0.17 CFU/mL (T1) at 24 hours. At 48 hours, it ranged from Log_{10} 0.10 (T3) to Log_{10} 0.17 CFU/mL (T1), and Log_{10} 1.16 (T3) to Log_{10} 3.78 CFU/mL (T6) at 72 hours. At 24 hours, the PM was highest in T1 (96.6±0.5%) and least in T2 (61.6±2.8%). At 48 hours, the PM was highest in T1 (89.7±0.6%) and least in T2 (58.3±2.9%). Acrosome integrity was similar across all observation hours. The pH was similar across all observation hours ranging from 6.8 (T1) to 7.1 (T6), respectively. Boar semen extension using *Ocimum gratissimum* leaf extract at 0.5% resulted in reduced bacterial activities till 48 hours at 17°C.

Keywords: Antimicrobial activities, *Ocimum gratissimum* leaf extract, Porcine semen, Acrosome Integrity, Artificial Insemination.

INTRODUCTION

Liquid storage of boar semen usually at 17 °C is a conventional method used for Artificial Insemination (AI) in pig production, it is a traditional tool for reproduction and the preferred method in the porcine industry which may be adversely influenced for a range of reasons, including the contamination of ejaculates by various genera of microorganisms at different concentration levels (Mazurova *et al*., 2015; Alfonso *et al*., 2017). Bacterial contamination of extended semen is a major limitation in achieving high success rates of artificial insemination (AI) in swine production and this contamination has been associated with spermatozoa agglutination, reduced spermatozoa motion activity, it is also further associated with alterations to membrane and acrosome thereby rendering the affected semen samples to be less efficient in accomplishing fertilization. (Althouse and Lu, 2005; Althouse *et al*., 2008; Maroto Martin *et al*., 2010; Gaczarzewicz *et al*., 2016; Waberski *et al*.,2019; Menezes *et al*., 2020). The presence of these microbes in semen is unavoidable because semen collection is not a sterile process, though strict hygiene measures are taken during semen collection. (Alfonso *et al*., 2017). This therefore requires that antibiotics be added to commercial semen extenders for artificial insemination to inhibit microbial growth. Despite achieving a reduction in bacterial growth, with the use of

conventional or synthetic antibiotics, many antibiotics harm spermatozoa survival. Therefore, a combination of several antimicrobial agents at low concentrations is recommended. However, this non-therapeutic use of antibiotics in smaller doses can still lead to the development of antimicrobial resistance (Morrell and Wallgren 2014). There is therefore a need to decrease antibiotic use or to replace synthetic antimicrobial agents added to semen extenders. One of the possible alternatives may be the utilisation of natural substances with antimicrobial properties. One such promising natural substance is Scent leaf, scientifically known as *Ocimum gratissimum*, which belongs to Lamiaceae family. It is commonly found in tropical regions of Africa and Asia, and it is known by various names such as clove basil, African basil, and effirin in Nigeria. Scent leaf has a distinctive aroma and is widely used as a culinary herb and for medicinal purposes. (Talabi and Makanjuola, 2017) In traditional medicine, scent leaf has been used for centuries due to its various medicinal properties. It is believed to possess antimicrobial, anti-inflammatory, analgesic, and antioxidant effects. The leaves of the plant are rich in bioactive compounds such as phenols, flavonoids, alkaloids, and essential oils, which contribute to its therapeutic potential. (Ugbogu *et al*., 2021). The antimicrobial activity of scent leaf has been a subject of interest in scientific research. Authors (Ugueri *et al*, 2015; Agholar *et al*., 2018; Unegbu *et al*., 2019) have investigated its efficacy against various bacterial strains, with the paucity of studies on the use as boar semen in the hot, humid tropical environment. This study was aimed at investigating the *in vitro* antibacterial efficacy of *Ocimum gratissimum* leaf extract on boar semen.

MATERIALS AND METHODS

Collection of plant materials:

Fresh, healthy and uninfected scent leaves were harvested at the Teaching and Research Farm, University of Ibadan, located at Latitude $07^{0}26^{'}$N and Longitude $03^{0}54^{'}$E with elevation ranging from 150 m to 275 m above sea level.

Experimental Site

Semen collection was carried out at the Piggery Unit of the Teaching and Research Farm, University of Ibadan, Nigeria, located at Latitude $07^{0}26^{'}$N and Longitude $03^{0}54^{'}$E with elevation ranging from 150 m to 275 m above sea level. The semen analysis was carried out at the Animal Physiology Laboratory Unit, Department of Animal Science, University of Ibadan, Ibadan, Nigeria.

Experimental Animal and Management

A matured boar aged 3 years old weighing 250kg was used for this study. All routine and occasional management practices were carried out as and when due. The boar was provided with clean water and fed *ad libitum* throughout the study.

Semen Collection and Pre-extension Evaluation

Semen samples were collected using the gloved hand method. Before semen collection, the boar was thoroughly washed to remove urine and other materials that could increase the chances of semen contamination during collection. As the boar initiates extension of the penis, the end of the penis, which is corkscrew shaped was grabbed firmly with a gloved hand. The collection process was initiated with firm pressure on the spiral end of the penis with the hand so the penis could not rotate in the hand. The collection continued until the boar dismounted. The semen sample collected was transferred to the Animal Physiology Laboratory, Department of Animal Science, University of Ibadan, Ibadan where semen samples were immediately evaluated for colour, mass activity, progressive motility and pH.

Upon arrival at the Laboratory, the pH and temperature of the semen sample were determined using an MW802pH /EL/TDS/meter and thermometer, respectively. The pH meter was calibrated according to the manufacturer's instructions. Three-point calibrations were performed using buffers of pH 4.0, 7.0 and 9.0

Motility was determined by dropping (10 µl) of undiluted semen on a warmed (37 °C) microscope slide overlaid with a cover slip and observed for

sperm motility by phase contrast microscope at X400 magnification in at least four fields on the slide taking the average of these readings to obtain the final motility estimate.

Semen Collection:

Semen was collected using the gloved-hand technique into an insulated thermos US semen cup covered with gauze to filter out the gel portion to collect the sperm-rich portion. The collected semen sample was evaluated immediately for colour, mass activity, progressive motility and pH.

Isolation and Identification of Bacteria in Raw Boar Semen

Preparation of media:

The media used for the isolation of microorganisms were MacConkeyagar, *Salmonella-shagella* agar, Potato Dextrose agar, Plant count agar, Man Rogosasharpe. These media were prepared according to the manufacturer's instructions. The solution was homogenized in a water bath for 10 minutes, sterilized in an autoclave at 121 °C for 15 minutes and allowed to cool at 35 °C

Serial dilutions:

The 9 mL of distilled water (sdH_2O) was pipetted into the clean test tube and was covered with cotton wool and foil and was autoclaved at 121 °C for 15mins. The pour plate method was used to plate out the samples. A sterile pipette was used to transfer a mL of each semen sample into 9mL of sterile distilled water, shaken properly to obtain 10^{-4}dilutions. Precisely a mL of each dilution was discharged into the center of the appropriate petri dish. The plates were then incubated at 35 ^{0}C for 24 hours and observed for growth of micro-organisms.

Antimicrobial Activities of *Ocimum gratissimum* Leaf Extract

Bacterial isolation:

The bacteria isolated were cultured in peptone water for 18 h. 0.3 ml of each of the bacterial suspensions was mixed with 15 ml of MacConkey agar in sterile petri plates and allowed to solidify. A sterile steel borer was used to punch wells into the agar and each well was filled in with 0.1 mL extract (0.0, 0.5, 1.0, 1.5 and 2.0g/L). Penicillin and streptomycin were used as the positive control. Petri dishes were incubated at 37 °C for 24 hours and the diameter in millimeters of the zone of growth inhibition was measured using a transparent ruler.

Semen Collection:

Semen was collected using the gloved-hand technique into an insulated thermos US semen cup covered with gauze to filter out the gel portion to collect the sperm-rich portion. The collected semen sample was evaluated immediately for colour, mass activity, progressive motility, pH, normal cell and livability.

Colour was evaluated by visual observation. The consistency was scored as 1 = watery cloudy, 2 = milky, 3 = thin creamy, 4 = creamy and 5 = creamy-grainy (Shamsuddin *et al*., 2000).

To evaluate the mass activity (wave motion) a drop (10 µl) of undiluted semen was placed on a pre-warmed slide at 37 °C covered with a cover slip and examined under a phase contrast microscope (400× magnification). The mass activity was scored 0 = no motility, 1 = few sperm with weak movement (<20%), 2 = some motile spermatozoa (20–40%) without wave movement, 3 = slow wave movement (40–60%) with motile spermatozoa, 4 = rapid wave movement without whirlpool (60–80%) with motile spermatozoa and 5 = very rapid wave movement with clear whirlpools (>80%) motile spermatozoa (Avdi *et al*., 2004).

The sperm motility was estimated subjectively by preparing a wet mount of undiluted semen by placing a 10µl drop of fresh semen under a cover slip with the magnification of 400× using a phase contrast microscope.

The pH and temperature of the semen sample were determined using MW 802pH /EL/TDS/meter and thermometer respectively. The unit was calibrated according to the

manufacturer's instructions. Three-point calibrations were performed using buffers of pH 4.0, 7.0 and 9.0.

Semen Processing

Semen aliquots were diluted in each of the six experimental extenders. Diluted semen was preserved in a thermo-regulated fridge at 17 °C and evaluated for quality at 0, 24 and 48 h.

Microbial Count

The viable bacterial count was determined by dissolving 23g nutrient agar (Gilco lab USA) in 1000 mL of distilled water and boiling to boiling point. The solution was autoclaved at 121°C for 20 min. Sterilized petri dishes having at least 85 mm inside diameter were used. 0.5 mL semen samples were poured into each petri dish. The carefully closed petri dishes were incubated at 32 ºC for 48 h. Manual counting was performed with the aid of magnification under uniform and properly controlled artificial illumination (APHA, 1992). All the colonies are counted including those of pinpoint size. Serial dilutions were prepared and viable numbers were enumerated using the pour plate technique. Replicates of four dishes for each treatment and control sample were performed. Serial dilutions were prepared and viable numbers were enumerated using the pour plate technique. The results were expressed as Log_{10}CFU/mL according to, the American Public Health Association (APHA) (1992).

Semen Quality Assessment

Extended semen samples were reactivated in a water bath at 35 °C for 30min before evaluating for quality which was performed at 0, 24 and 48 hours as described above.

Experimental Treatment Allotment

Treatment 1. 4mL BTS(a) + 1mL Semen
Treatment 2. 4mL BTS(b) + 1mL Semen
Treatment 3. BTS(b) + 0.5% OgLE (0.2mL OgLE + 3.8mL BTS(b) + 1mL Semen
Treatment 4. BTS(b) + 0.1% OgLE (0.4mL OgLE + 3.6mL BTS(b) + 1mL Semen
Treatment 5. BTS(b) + 1.5% OgLE (0.6mL OgLE + 3.4mL BTS(b) + 1mL Semen
Treatment 6. BTS(b) + 2.0% OgLE (0.8Ml OgLE + 3.2mL BTS(b) + 1mL Semen
BTS= Beltsville Thawing Solution
BTS(a)+ Antibiotics (Streptomycin (1.1g/L) and Penicillin(1.1g/L)
BTS(b)- Antibiotics
OgLE= *Ocimum gratissimum* Leaf Extract

Table 1: Chemical composition of Beltsville Thawing Solution (BTS) extender

Composition	Quantity(g/L)
Glucose	37.00
Sodium citrate	6.00
Potassium chloride	0.75
EDTA	1.25
Sodium bicarbonate	1.25
Penicillin	1.1
Streptomycin	1.1

Source: Johnson, 2000

EDTA: Ethylenediamine tetra acetate

Statistical analysis: Data were subjected to descriptive statistics and one-way analysis of the variance procedure of SAS (2003) and means were separated using Duncan's multiple range test of the same software at $\alpha_{0.05}$.

RESULTS

Percentage occurrence of Microbes

The different bacteria species isolated from boar semen are presented in Table 2. Two bacteria species were isolated from the ejaculate of boar collected for this investigation. The bacteria species isolated with their frequency of occurrence were (20.2%) *Salmonella* species and (30.1%) *Escherichia coli*. The predominant bacteria species was *Escherichia coli* in the ejaculate of boar semen.

Table 2: Percentage occurrence of Microorganisms isolated from boar semen

Species	Number of occurrences	Percentage (%)
Salmonella shigella	2	20.2
Escherichia coli	3	30.1
Lactobacillus	ND	----

ND= Not Detected

Figures 1, 2 and 3 reveal the mean values obtained for the percentage progressive motility of boar spermatozoa extended with OgLE at 17 ^{0}C. At 0hour significant effect ($p< 0.05$) was observed with T1 having the highest value (98.00%) which is significantly similar to the value obtained at 0.5g/L inclusion of OgLE (97.67%) T3. Mean values obtained for T2 and T4 BTS minus antibiotics and 1.5g/L respectively were significantly ($p>0.05$) similar (97.00%) and (97.00%). At 24 hours post preservation significant difference ($p<0.05$) was observed with T1 having the highest value at 96.00% while 0.5g/L, 1.0g/L, 1.5g/L and 2.0g/L were significantly similar ($p>0.05$) with T2 having the lowest value (83.00%). A significant difference ($p<0.05$) was observed at 48 hours post-storage with T3 having the highest value (90.33%) and T2 having the lowest value (79.33%).

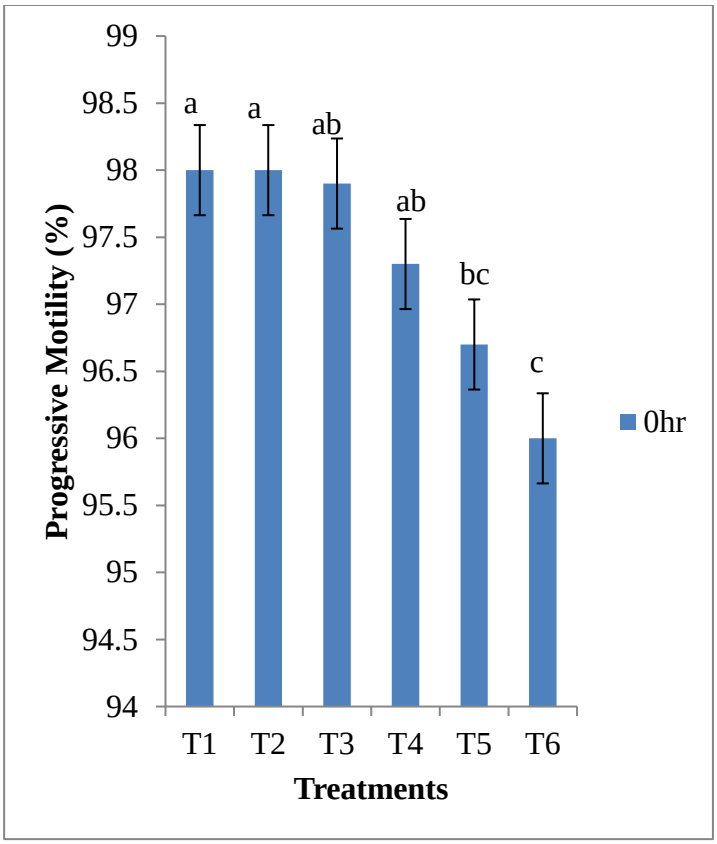


Figure 1: Effect of OgLE on percentage progressive motility of extended boar semen for 0 hours at 17 °C.
T1= BTS(A)
T2= BTS(B)
T3= 0.5% OgLE
T4= 1.0% OgLE
T5= 1.5% OgLE
T6= 2.0% OgLE

BTS (A): Beltsville Thawing Solution with antibiotics
BTS (B): Beltsville Thawing Solution without antibiotics
OgLE= *Ocimum gratissimum* Leaf Extract

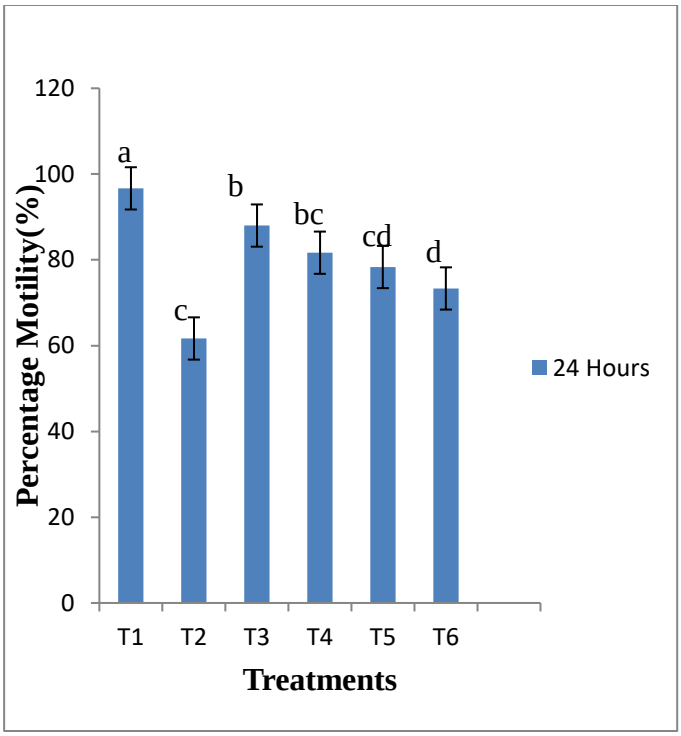


Figure 2: Effect of OgLE on percentage progressive motility of extended boar semen for 24 hours at 17 °C.
T1= BTS(A)
T2= BTS(B)
T3= 0.5% OgLE
T4= 1.0% OgLE
T5= 1.5% OgLE
T6= 2.0% OgLE

BTS (A): Beltsville Thawing Solution with antibiotics
BTS (B): Beltsville Thawing Solution without antibiotics
OgLE= *Ocimum gratissimum* Leaf Extract

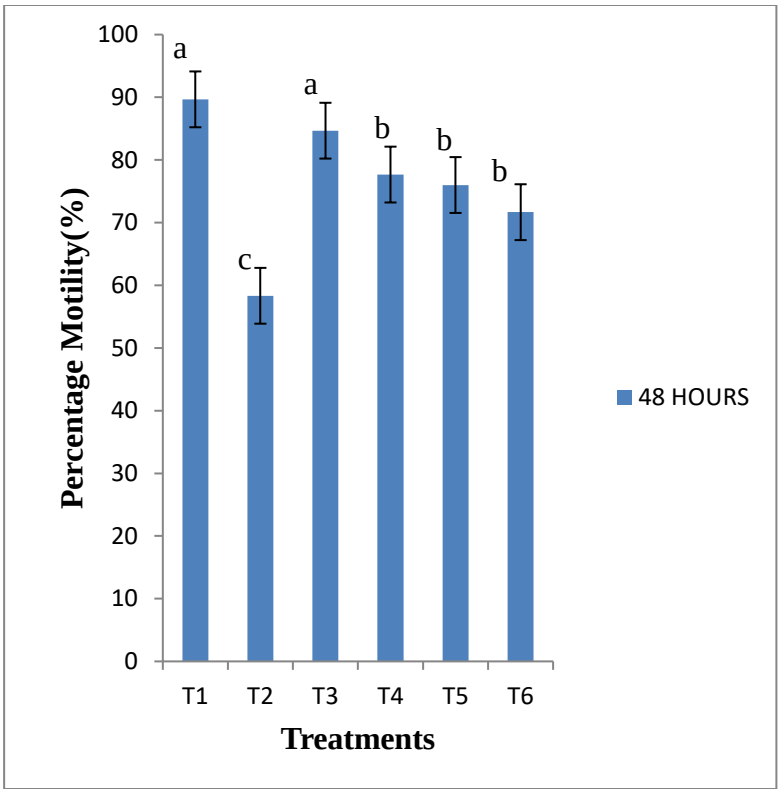


Figure 3: Effect of OgLE on percentage progressive motility of extended boar semen for 48 hours at 17 °C.
T1= BTS(A)
T2= BTS(B)
T3= 0.5% OgLE
T4= 1.0% OgLE
T5= 1.5% OgLE
T6= 2.0% OgLE

BTS (A): Beltsville Thawing Solution with antibiotics
BTS (B): Beltsville Thawing Solution without antibiotics
OgLE= *Ocimum gratissimum* Leaf Extract

Table 3 shows the pH of semen in an extender having OGLE in different concentrations as a source of antibiotics. It was observed that at 0-hour pH values obtained ranged between 6.87 (T3 and T4) to 7.00 (T2) with significant ($p < 0.05$) difference in all the treatment means. At 24 hours post storage, no significant effect ($p > 0.05$) among the treatment means with T2 and T5 having the highest pH values of 7.07 and 7.07, while T3 had the lowest value of 6.93. pH values obtained at 48 hours indicated significant difference($p < 0.05$) among treatment means.

Table 3: Effects of *Ocimum gratissimum* Leaf Extract on the pH of Boar Semen at 17 °C

Treatments	T1	T2	T3	T4	T5	T6
0 hour	6.97±0.06^{ab}	7.00±0.00^{a}	6.87±0.06^{c}	6.87±0.06^{c}	6.93±0.06^{abc}	6.90±0.00^{bc}
24 hours	7.00±0.10	7.07±0.06	6.93±0.06	7.00±0.17	7.07±0.06	6.97±0.06
48 hours	6.93±0.06^{bc}	6.90±0.00^{c}	6.97±0.06^{abc}	7.03±0.06^{ab}	7.07±0.06^{a}	7.03±0.06^{ab}

$^{(a, b, c,)}$ Means along the same row with different superscripts are significantly ($p<0.05$)

Presented in Table 4 is the effect of OgLE on the percentage acrosome Integrity of boar semen at 17 °C. The percentage acrosome integrity values obtained ranged from 95.67% (T6) to 97.67% (T1). Treatment T1 was significantly different ($p<0.05$) from other treatments, while T2 (96.67%) to T6 (95.67%) were statistically similar ($p>0.05$). At 24 hours, there were significant differences ($p<0.05$) across the mean values obtained. Treatments T2 (92.00%) and T3 (90.00%) were significantly the same also, T5 (90.00%) and T6 (90.00%) were significantly similar ($p>0.05$). At 48 hours of post-preservation, T2 (92.67%) had the highest value and T1 (90.33%), T3 (90.67%), T4(89.67%), T5(89.33%) and T6 (89.33%) were statistically similar ($p>0.05$).

Table 4: Effects of *Ocimum gratissimum* Leaf Extract on the Acrosome Integrity of Boar Semen at 17 °C

Treatments	T1	T2	T3	T4	T5	T6
0 hour	97.67±0.58^{a}	96.67±0.58^{b}	96.67±0.58^{b}	96.33±0.58^{b}	96.00±0.00^{b}	95.67±0.58^{b}
24 hours	95.00±0.00^{a}	92.00±1.73^{b}	92.33±1.53^{b}	91.00±0.00^{bc}	90.00±0.00^{c}	90.00±0.00^{c}
48 hours	90.33±0.58^{b}	92.67±2.08^{a}	90.67±0.58^{b}	89.67±0.58^{b}	89.33±0.58^{b}	89.33±0.58^{b}

$^{(a, b, c,)}$ Means along the same row with different superscripts are significantly ($p<0.05$)

Microbial Load ($x10^5$cfu/mL) of Boar Semen Extended with Scent Leaf Extract at 17 ºC is presented in Figure 5. At 24 hours, the least bacterial load was observed in T2 ($0.03x10^5$cfu/mL), followed by T5, 1.5% OgLE ($0.07x10^5$cfu/mL), T3, 0.5% OgLE ($0.13x10^5$cfu/mL), T1 ($0.23x10^5$cfu/mL), T4, 0.1% OgLE ($0.43x10^5$cfu/mL), and T6, 2.0% OgLE ($0.67x10^5$ cfu/mL). At 48 hour, no growth was observed in treatments T4, 0.1% OgLE ($0.00x10^5$cfu/mL), T5, 1.5% OgLE ($0.00x10^5$cfu/mL) and T6, 2.0% OgLE ($0.00x10^5$cfu/mL), followed by T3, 0.5% OgLE ($0.01x10^5$cfu/mL), T1 ($0.04x10^5$cfu/mL) and highest bacterial load of $0.06x10^5$cfu/mL was recorded at T2.

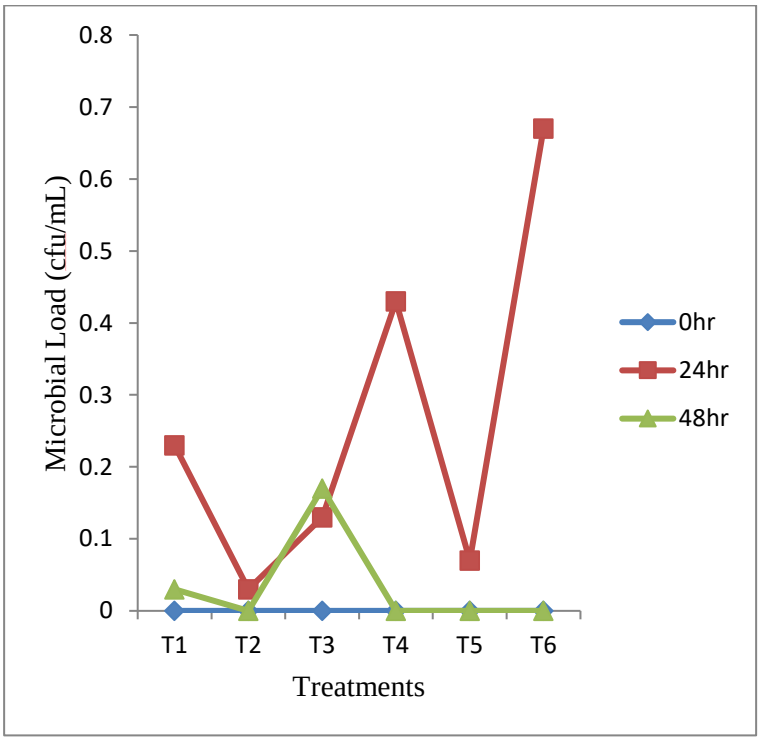


Figure 5: Effect of OgLE on microbial load of extended boar semen at 17 °C.
T1= BTS(A)
T2= BTS(B)
T3= 0.5% OgLE
T4= 1.0% OgLE
T5= 1.5% OgLE
T6= 2.0% OgLE

BTS (A): Beltsville Thawing Solution with antibiotics
BTS (B): Beltsville Thawing Solution without antibiotics

DISCUSSION

Occurrence of microbes isolated from boar semen

Although well known for its nutritive value, *Ocimum gratissimum* (scent leaf) has been found to have the potential to exhibit microbial growth. (Chassagne *et al*., 2021). This study observed that the ethanolic and aqueous extracts of *Ocimum gratissimum* have some antibacterial activity, The mechanism of the antimicrobial actions of phenolic compounds including scent leaf extract is based mainly on their ability to disrupt the integrity of the bacteria cytoplasmic membrane and to interfere with the bacteria metabolism and proteosynthesis (Cristani *et al*.,2007; Xu *et al*.,2008; Oyedemi *et al*.,2009; Devi *et al*.,2010; Silva and Fernandes 2010; Hyldgaard *et al*.,2010 and Mazurova *et al*.,2015. Many factors directly affect the quality and storage of spermatozoa, one of the most important of which is bacterial contamination is referred to as bacteriospermia (Sanchez, 2016). The majority of contaminants are gram-negative bacteria, and most of them

belong to the *Enterobacteriaceae* family (Althouse and Lu, 2005; Althouse and Rossow, 2011). Bacterial contamination is a serious and very real deterrent to profitable semen production in swine. It is routinely observed in raw and extended semen produced for artificial insemination (AI) when semen is collected by the gloved-hand technique Bresciani *et al*., 2014.
The result of the percentage occurrence of micro-organisms isolated from raw boar semen was presented in Table 2. The result revealed that *Escherichia coli* was the most frequently isolated (30.1%) followed by *Salmonella shigella* (20.2%) while *Lactobacillus* was not detected. This result agrees with the findings of Bresciani *et al.*, 2014 who reported that out of all the isolates detected from boar semen samples, *E. coli* was the most frequent contaminant bacterium (46.5%) followed by *Staphylococcus epidermidis* (18.6%), *Serratia marcescens* (13.95%), *Staphylococcus aureus and Streptococcus spp* (4.65% each), and *Pseudomonas spp*. (2.3%). Also, Althouse and Lu (2005) as well identified 25 different genera of bacteria in boar semen among which were *E. coli*, *Pseudomonas*, *Staphylococcus* and *Proetus spp*. The reason is that semen is an ideal medium for the establishment and growth of many micro-organisms including bacteria (Lombardo and Thorpe, 2000). Furthermore, Lombardo and Thorpe (2000) also isolated *E. coli, Salmonella spp; Shigella* spp; and *Yersinia* spp; in swallow semen, while Mangiagalli *et al*., (2012) and Martino *et al*., (2006) isolated only *E.coli* and *S.faecalis* in rabbit and rooster semen respectively. Meanwhile, the isolation of *E. coli,* and *Salmonella shigella* in the present study is significant due to their deleterious effects on viability of spermatozoa. *Escherichiacoli* induces an agglutinating effect on 40 to 75% of motile spermatozoa, as reported by Althouse (2008). Bacterial contamination overgrowth yields worse semen quality and longevity (Aurich and Spergser, 2007). The presence of microorganism, especially bacteria in an ejaculate can affect fertilization directly, by adhering to spermatozoa thereby impairing their motility (Khalid *et al*., 2010).

Effects of Ocimum gratissimum leaf extract on the microbial load and spermatozoa quality evaluation of boar semen at 17 ^{o}C

Quality evaluation of boar semen extended with scent leaf extract at 17 ^{o}C

The number of motile spermatozoa, particularly those with progressive motility, is one of the most frequent measures used to estimate the quality of an ejaculate, given its importance for spermatozoa migration through the female genital tract and penetration of the oocyte membrane (Maria *et al*.,2014). This makes it to be considered to be one of the factors determining *in vitro* fertilisation rates. (Simon and Lewis, 2011; Turner 2006). Thus, in this present study, the inclusion of different concentrations of OgLE in the BTS extender showed that at collection time, the motility of spermatozoa was vigorous and progressive. It was observed that progressive motility of the sperm cell was greater than 90% across the treatments at 0 hours, this is similar to the findings of Khan *et al*.,2006 who reported that average total motility and progressive forward motility of boar spermatozoa preserved in two different extenders (BTS and SAFEcell plus) was greater than 90% in both extenders on the day 0 of storage.

The inclusion of different concentrations of OgLE in boar semen extender had no detrimental effect on spermatozoa motility at 0, 24 and 48 hours of preservation. This could be attributed to the ability of the OgLE in the diluent to serve as an antimicrobial needed to inhibit the growth of micro-organisms that could detrimentally affect the survival of sperm cells. This result supported the observations of Agatemor, 2005; Lopez *et al*.,2005 and Ladipo *et al*.,2010, these authors reported that OgLE has antimicrobial properties that could inhibit the growth of micro-organisms. It was also observed that at 24 hours, progressive motility decreased though not significantly

because all the treatments had a percentage progressive motility above 60%, and this is per the observation of John *et al*., 2003 who rated spermatozoa motility between 50 and 70% as good motility. At 48 hours, semen extended with 0.5% OgLE competed favourably with positive control while, negative control had percentage motility below 60% though still within the recommended range reported by Johnson *et al*., 2003. At this point, the different concentrations of OgLE (0.5, 1.0, 1.5 and 2.0%) still sustained motility of the sperm cells at 60%. Furthermore, Coy *et al*., (2009) used a plant extract to increase the viscosity of a medium for swine spermatozoa and observed an increase in the percentage of progressive motile spermatozoa.

One way to evaluate semen quality is by determining the acidic level of the semen.
Here, the mean pH values obtained in all the treatments throughout the preservation periods were minimal. They were within the normal physiological range (6.8-7.2) for boar semen as reported by Paulenze *et al*., (2000). The pH of the semen at collection time ranged from 7.00 - 7.02. The inclusion of different concentrations of OgLE in the BTS extender yielded mean values of pH that are within the acceptable range which agrees with the findings of Frunza *et al*., (2008) who reported that a pH value that is higher than 8 is an indication of poor-quality semen. It can further be said that OgLE in BTS extender acts more efficiently as a buffer which also makes the spermatozoa benefit from a more homogenous distribution of different concentrations of OgLE in the extender according to Nagy *et al*., (2002).

Acrosome integrity is a vital parameter for predicting spermatozoa's fertilising capacity *in vitro* (Sokunbi *et al*.,2020). This study observed that acrosome integrity was intact across the treatment from the time of collection up to 48 hours. Suffice it to say that including different concentrations of OgLE in the boar semen extender sustained the integrity of the spermatozoa acrosome. Acrosome integrity was above 80% in all the treatments throughout the storage time and so was said to be good and intact, and this is justified by the findings of Maes *et al*., (2010) who reported that semen samples with less than 70% sperm with intact acrosome should be discarded before processing.

The presence of bacteria in extended semen creates competition for nutrients and also results in the production of metabolic by-products that may harm the spermatozoa (Althouse, 2008). In this study, different concentrations of OgLE were added to the boar semen extender, and it was observed that all the different concentrations inhibited the growth of bacterial colonies across the treatments from 0 – 48 hours of preservation. At 0 hours, no visible bacteria growth was detected except for insignificant growth in both the positive and negative control treatments and 0.5% OgLE inclusion. This implies that the inclusion of different concentrations of OgLE inhibited the growth of micro-organisms. This is justified by the findings of Ekhaise *et al*.,2010 who reported that scent leaf extract contains antibacterial properties that could inhibit the growth of bacteria.
The mechanism of the antimicrobial actions of phenolic compounds including scent leaf extract is based mainly on their ability to disrupt the integrity of the bacteria cytoplasmic membrane and to interfere with the bacteria metabolism and proteosynthesis (Cristani *et al*.,2007; Xu *et al*.,2008; Oyedemi *et al*.,2009; Devi *et al*.,2010; Silva and Fernandes 2010; Hyldgaard *et al*.,2010 and Mazurova *et al*.,2015.

CONCLUSION

The addition of OGLE in the BTS extender for its potential antibacterial properties showed that motility, pH, and acrosome integrity were not adversely affected at 17 ºC for 48 hours at 0.5% inclusion. It was demonstrated in this study that boar semen is not free of contamination, especially bacteria, no matter the physiological state of the donor. However, this suggests the adverse effects of bacteria on fertilisation either

directly or indirectly. Thus, the inclusion of different concentrations of OGLE inhibited and reduced the growth of bacteria in extended boar semen.

REFERENCES

Agatemor, C. 2005. Antimicrobial activity of aqueous and ethanol extracts of nine Nigerian spices against four food borne bacterial. *Elec. J. Environ. Agric. Food Chem*. 8(3): 195-200.

Agholor K, Yaki LM, Abubakar I, Olusola LF, Rakiya Z. 2018 Antibacterial activity of Ocimum gratissimum (scent leaf) on some pathogenic gastrointestinal bacteria. *Afr J Microbiol Res*. 12(40): 923-929. doi: 10.5897/AJMR2018.8847

Alfonso L. Rz., Ann V. Sm., and D. Ms 2017. Boar management and semen handling factors affect the quality of boar extended semen. Porcine Health Management

Althouse, G. C. 2008. Sanitary procedures for the production of extended semen. *Reproduction in Domestic Animals* 43, 374-378.

Althouse, G. C. and Lu K. G. 2005. Bacteriospermia in extended porcine semen. *Theriogenology* 63: 573-584.

Althouse, G. C. and Rossow K. 2011. The potencial Risk of Infectious Disease Dissemination Via Artificial Insemination in Swine. *Reprod Dom Anim* 2:64-67.

American Public Health Association (APHA) (1992).

Avdi, M., Leboeuf, B. and Terqui, M. 2004. Advanced breeding and buck effect in indigenous Greek goats. *Livestock Production Science,* 87: 251-257.

Chassagne F, Samarakoon T., Porras G., Lyles J. T, Dettweiler, M and Quave C. L, 2021. A Systematic Review of Plants with Antibacterial Activities: A Taxonomic and Phylogenetic Perspective. Front Pharmacol. 11: 1-29.

Coy, P., Gadea, J., Rath,D. and Hunter, R. H. 2009. Differing sperm ability to penetrate the oocyte in vivo and in vitro as revealed using colloidal preparation. *Theriogenology,* 72(9), 1171-1179. http://dx.doi.org/10.1016/j.theriogenology.

Cristani, M., D'Arrigo M., Mandalari G., Castelli, F., Sarpietro, M. G., Micieli, D., Venuti, V., Bisignano, G., Saija A., Trombetta D. 2007. Interaction of four monoterpenes contained in essential oils with model membranes: Implications for their antibacterial activity. *Journal of Agricultural and Food Chemistry* 55, 6300-6308.

Devi, K. P., Nisha S. A., Sakthivel, R., Pandian, S. K. 2010. Eugenol (an essential oil of clove) acts as an antibacterial agent against *Salmonella typhi* by distrupting the cellular membrane. *Journal of Ethnopharmacology* 130, 107-115.

Ekhaise, F. O, Soroh, A.E. and Falodun, A. 2010. Antibacterial properties and preliminary phytochemical analysis of Methanolic extract of *Ocimum gratissimum* (Scent leaves). *Bayero Journal of Pure and Applied Sciences*, 3(2): 81-83.

Gączarzewicz D., Udała J., Piasecka M., Błaszczyk B., Stankiewicz T. 2016. Bacterial Contamination of Boar Semen and its Relationship to Sperm Quality Preserved in Commercial Extender Containing Gentamicin Sulfate. *Pol. J. Vet. Sci.* 19:451–459. doi: 10.1515/pjvs-2016-0057. [PubMed] [CrossRef] [Google Scholar] [Ref list].

Hyldgaard, M., Mygind, T. and Meyer, R. L. 2012. Essential oils in food preservation: Mode of action, synergies, and interactions with food matrix components. Frontiers in Microbiology 3, 1-24.

Johnson, R. C., Douglas, K. M. and Karen, L. C 2003. Animal Science: The biology, care and production of domestic animals. Mc Graw Hill Higher Education. Fourth edition. Pp 70-72.

Khan, M. H., Naskar, S., Anubrata Das, Bardoli R. K. 2006. Comparative efficacy of different diluents on liquid preservation of boar semen. India *J. Anim Sci*. 76(10): 780-783.

Ladipo, M. K., Doherty, V. F. and Kanife, U. C. 2010. Phytochemical Screening and Antibacterial Investigation of The Extract of *Ocimum gratissimum* (Scent leaves) on Selected *Enterobacteriaceas*. PAT. 6(2): 75-84.

Lombardo, M. P. and Thorpe, P. A. 2000. Microbes in tree swallow semen. *J. Wild. Dis*. 36(3): 460-468.

Lopez, P., Sanchez, K., Batlle, R and Nerin, C. 2005. Solid and vapor phase antimicrobial activities of six essential oils susceptibility of selected food bornebacterial and fungal strains. *J. Agric Food Chem*. 53 (17): 6939-6946.

Maes, D., Rijsselaere, T., Vyt, P. H., Sokolowska, A.,Deley, W. and Van Soom, A. 2010. Comparison of five different methods to assess the concentration of boar semen. Vlaams Diergeneskundig Tijdschrif 79: 42-48.

Mangiagalli, M. G., Cesari, V.,Cerolini, S., Luzi, F. and Toschi, I. 2012. Effect of lucopene supplementation on semen quality and reproductive performance in rabbits. *World Rabbit Sci*. 20: 141-148.

Maria, C. C. A., Francisco, J. B., Jesus, M. G., Luis, J., Garcia-Marin and Javier, G. 2014. Increasing extender viscosity improves the quality of cooled boar semen. *Journal of Agricultural Science;* Vol. 6, No.3.

Maroto Martin, L. O., Munoz, E. C., De Cupere, F., VanDriessche, E., Echemendia-Blanco, D., Machado Rodriguez, J. M. and Beeckmans, S. 2010. Bacterial contamination of boar semen affects the litter size. *Anim. Reprod. Sci*. 120: 95-104.

Martino, P. A., Mangiagalli, M. G., Marelli, S. P., Smajlovic, T., Cavalchini, I. G. 2006. Effect of lycopene on semen bacteriological status and native immunity in roosters. *World Poult Sci. J*. 62(Suppl.): 415-419.

Mazurova, J., Kukla, R., Rozkot, M., Lustykova, A., Slehova, E., Sleha, R., Lipensky, J. and Opletal, L. 2015**.** Use of natural substances for boar semen decontamination. *Veterinarni Medicina*, 60 (5) : 235-247.

Menezes T.A., Mellagi A.P.G., da Silva Oliveira G., Bernardi M.L., Wentz I., Ulguim R. D. R., Bortolozzo F. P. 2020. Antibiotic-free extended boar semen preserved under low temperature maintains acceptable in-vitro sperm quality and reduces bacterial load. *Theriogenology*. 149:131–138. doi: 10.1016/j.theriogenology.2020.03.003. [PubMed] [CrossRef] [Google Scholar] [Ref list].

Morrell, J. M. and Wallgren, M. 2011. Control of bacterial contamination in boar semen doses. In: Mendez-Vilas A., editor. Science and Technology against Microbial Pathogens. World Scientific Publishing Co., Pte. Ltd.; Singapore, Singapore: pp. 303–308. Research, Development and Evaluation.

Oyedemi, S. O., Okoh, A. I., Mabinya, L. V., Pirochenva, G. and Afolayan, A. J. 2009. The proposed mechanism of bactericidal action of eugenol, α-terpineol and γ-terpinene against Listeria *monocytogenes, Streptococcus pyogenes, proteus vulgaris and Escherichia coli*. *African Journal of Biotechnology* 8, 1280-1286.

Paulenz, H., Kommisrud, E. and Hofmo, P. O. 2000. Effect of long-term storage at different temperatures on the quality of liquid boar semen. *Reprod. Dom. Anim*. 35: 83-85.

SAS. 2003**.** SAS STAT Usher's Guide Version 9.2 for windows. SAS Institute Inc., SAS Campus Drive, Cary, North Caroline, USA.

Silva, N. C. C. and Fernandes, J. A. 2010. Biological properties of medicinal plants: a review of their antimicrobial activity. *Journal of Venomous Animals and Toxins including Tropical Diseases* 16, 402-413.

Simon, L. and Lewis, S. E. 2011. Sperm DNA damage or progressive motility : which one is the better predictor of fertilization *in vitro*? *Systems Biology in Reproductive Medicine*, 57(3), 133-138.

Sokunbi, O. A., Ragland, D., Adeola, O., Adejumo, D. O., Adeshinwa, A., Ewuola, E. O., Adebiyi, O. A. and Olugasa, B. 2020. Artificial Insemination: A Critical Reproductive tool for moving the swine industry in Nigeria Forward. International Pig Summit Pp. 140-144.

Talabi J. Y, Makanjuola S. A. 2017. Proximate, Phytochemical, and In Vitro Antimicrobial Properties of Dried Leaves from *Ocimum gratissimum*. Prev Nutr Food Sci. 22(3): 191-194. doi:10.3746/pnf.2017. 22.3.191.

Turner, R. M. 2006. Moving to the beat: A review of mammalian sperm motility regulation. Reproduction, Fertility and Development, 18(1-2), 25-38. http//dx.doi.org/10.1071/RD05120.

Ugbogu O. C, Emmanuel O, Agi G. O, Ibe C, Ekweogu C. N, Ugbogu E. A. 2021. A review on the traditional uses, phytochemistry, and pharmacological activities of clove basil (*Ocimum gratissimum* L.). Heliyon. 7(11): e08404. doi: 10.1016/j.heliyon.20 21.e08404.

Ugueri U, Omeje F, Uloma I, Oseiwe F. 2015. Phytochemical Analysis of Vernonia Amygdalina and Ocimum gratissimum Extracts and their antibacterial activity on some drug resistant bacteria. *Am J Res Comm*. 3(5): 1-8.

Unegbu N. V, Obum-Nnadi C. N, Nkwoemeka N. E, Egwuatu P. I. 2019. Phytochemical and antibacterial activities of *Ocimum gratissimum* on some selected drug resistant bacteria. *Tr Sci Technol J*. 4(3): 786 – 789.

Waberski D., Luther A.M., Grünther B., Jäkel H., Henning H., Vogel C., Peralta W., Weitze K.F. 2019. Sperm function in vitro and fertility after antibiotic-free, hypothermic storage of liquid preserved boar semen. *Sci. Rep.* 9:14748. doi: 10.1038/s41598-019-51319-1. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

Xu, J., Zhou, F. J., B. P., Pei R. S. and Xu. N. 2008. The antibacterial mechanism of carvacrol and thymol against *Escherichia coli* Letter in Applied Microbiology 47, 174-179.