which the MBC were recorded as the lowest concentration giving no growth after culture. Three replicates were done for each extract concentration and controls.

2.5 Antimicrobial effect of basil essential oil in rice containing *Bacillus cereus:* *Bacillus cereus* strain 110 was chosen for this experiment because it seems to be the most resistant among the three.

To determine the antimicrobial effect of basil essential oil in cooked rice (with and without salt) spiked with *Bacillus cereus*, three concentrations (MBC, MIC, and ½ MIC) of basil essential oil was made in sterile distilled water. 10ml of an overnight broth culture was centrifuged at 6000rpm for 5 minutes and the supernatant discarded. The bacteria cells were re-suspended in sterile distilled water and centrifuged three times so as to wash all the nutrient broth away. The washed cells were re-suspended in 10ml sterile distilled water and a 1/100 dilution was made. 0.5ml washed cells + 0.5ml of plant extract was added to the rice samples. A control was also prepared in which 0.5ml cells + 0.5ml sterile distilled water instead of plant extract was used.

2.6 Statistical analysis: All experiments were performed in replicates. The results are expressed as mean ± standard deviation (SD) for the measurements of inhibition zones in antimicrobial activity tests. Data on cell viable count were statistically analysed using Microsoft excel 2013 and results presented as graphs.

3. Results

3.1 Antibacterial tests of basil (*Ocimum basilicum*) extract

3.1.1 Agar radial diffusion of basil essential oil: The results of this experiment showed that basil essential oil inhibited bacterial growth around wells containing neat, 50%, 25% and 12.5% (v/v) concentrations on all the tested bacteria (figure 1).

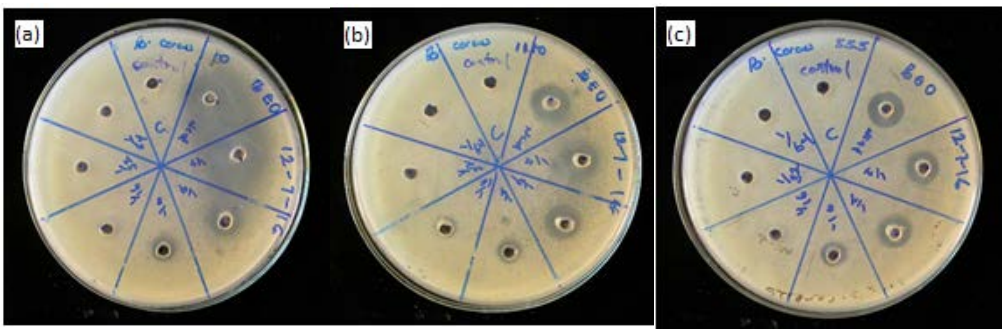

***Figure 1*:** agar radial diffusion results of basil essential oil (BEO) in 6mm wells against: (a) *B. cereus* 10; (b) *B. cereus* 110; (c) *B. cereus* 555 after incubation at 30^0 C for 24 hours.

Although all the tested organisms are of *Bacillus cereus* strains, there seems to be variations in growth inhibition. *B. cereus 10* seems to be more sensitive showing a zone of inhibition of 40 ± 3.1mm on the neat sample followed by *B. cereus* 555 with 23 ± 4.2mm while *B. cereus* 110 proves to be the most resistant with 22 ± 2.9mm zone of inhibition as shown in table 1.

Table 1: Agar radial diffusion results of basil essential oil in 6mm wells against selected *B. cereus* strains after incubation at 30^0 C for 24 hours. NI: no inhibition; n = 3.

Concentration (% v/v)	**Zone of inhibition diameter (mm) ± standard deviation**		
	B. cereus 10	***B. cereus* 110**	***B. cereus* 555**
Control	NI	NI	NI
1.56	NI	NI	NI
3.12	NI	NI	NI
6.25	NI	NI	NI
12.5	15 ± 0.9	13 ± 1	14 ± 0.6
25	21 ± 0.43	18 ± 0.43	15 ± 0.43
50	35 ± 1.8	20 ± 0.43	18 ± 1.6
Neat	40 ± 3.1	22 ± 2.9	23 ± 4.2

3.1.2 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) tests of basil essential oil: Table 2 shows that the MIC values of basil oil against *B. cereus* strain 10 and 555 appear to be the same at 0.097% (v/v). *B. cereus* 110 is the most sensitive having an MIC value of 0.048% (v/v). Despite having a different MIC value both *B. cereus* 10 and 110 have an MBC value of 0.097% (v/v). The MBC value of *B. cereus* 555 is at a slightly higher concentration of 0.195% (v/v).

Table 2: MIC and MBC values of basil essential oil against selected *B. cereus* strains after incubation at 30^0 C for 24 hours. N=2.

Bacteria	**Basil**	
	MIC (%v/v)	**MBC (%v/v)**
Bacillus cereus 10	0.097	0.097
Bacillus cereus 110	0.048	0.097
Bacillus cereus 555	0.097	0.195

3.1.3 Antimicrobial effect of basil essential oil in cooked rice containing *Bacillus cereus*: Experiments (fig. 2 and 3) were carried out to determine the antimicrobial activities of basil

essential oil in cooked rice samples with and without salt within 24 hours of incubation at 30^0C.

Figure 2 indicated at time 0, the number of cells are different for all 3 concentrations and control. This is because the antimicrobial activity of basil oil at all 3 concentrations started at time 0 with the MBC and control having the lowest and highest starting population respectively. Basil oil at MBC concentration reduced the number of bacterial cell count from 3.5 $\log_{10}$ cfu/ml to 1.1 $\log_{10}$ cfu/ml within 24hours. At the MIC concentration, there was a slight increase in microbial growth of 4.1 $\log_{10}$ cfu/ml to 4.8 $\log_{10}$ cfu/ml. An increase from 4.6 $\log_{10}$ cfu/ml to 6.6 $\log_{10}$ cfu/ml was observed at ½ MIC concentration. Despite the increase in bacterial growth at both MIC and ½ MIC, the results appear to be better than that of the control without extract of which the number of cells increased from 4.6 $\log_{10}$ cfu/ml to 9.2 $\log_{10}$ cfu/ml within 24 hours of incubation at 30^0C. Figure 2 indicated that basil oil affect the rate of growth of *B. cereus* 110 even at the low concentration of ½ the MIC.

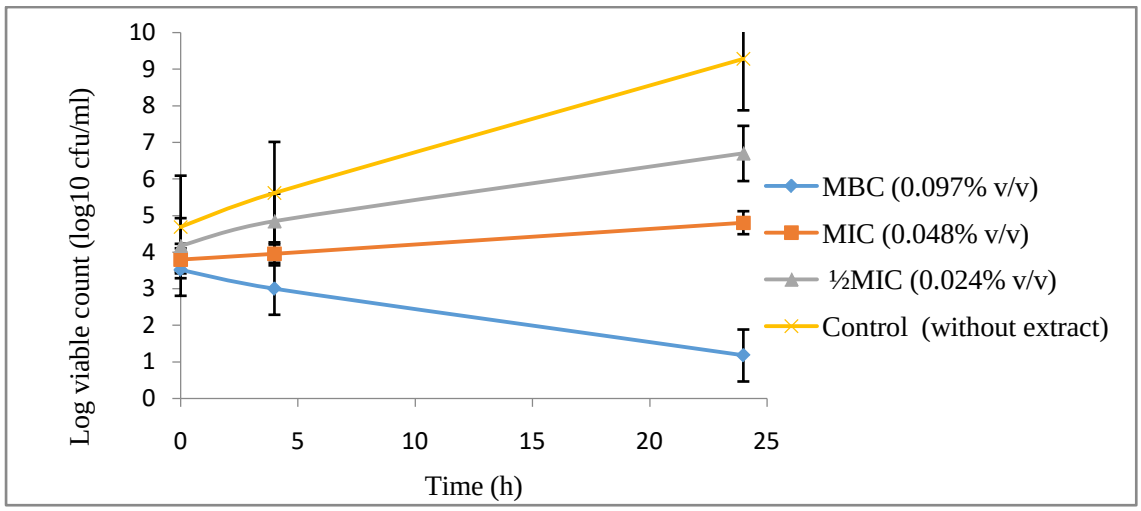

***Figure 2*:** Growth and survival of *B. cereus* (strain 110) in rice cooked without salt containing basil essential oil at the concentrations MBC, MIC, ½ MIC and control after incubation at 30^0C for 4 and 24 hours; N=3.

Furthermore, the results (figure 3) of antimicrobial activity of basil oil in rice containing salt (0.2%) does not show too much of a difference. The starting population is also 3.5 $\log_{10}$ cfu/ml at the MBC concentration but declined to 0.6 $\log_{10}$ cfu/ml within 24 hours of incubation at 30^0C. The MIC and ½ MIC at time=0 have a log count of 3.5 $\log_{10}$ cfu/ml and 3.8 $\log_{10}$ cfu/ml that increased to 4.6 $\log_{10}$ cfu/ml and 6.5 $\log_{10}$ cfu/ml respectively within 24hours. The control has an increase in bacterial cell count from 4.3 $\log_{10}$ cfu/ml to 8.9 $\log_{10}$ cfu/ml within 24 hours of incubation at 30^0C which is much higher than that of ½ the MIC.

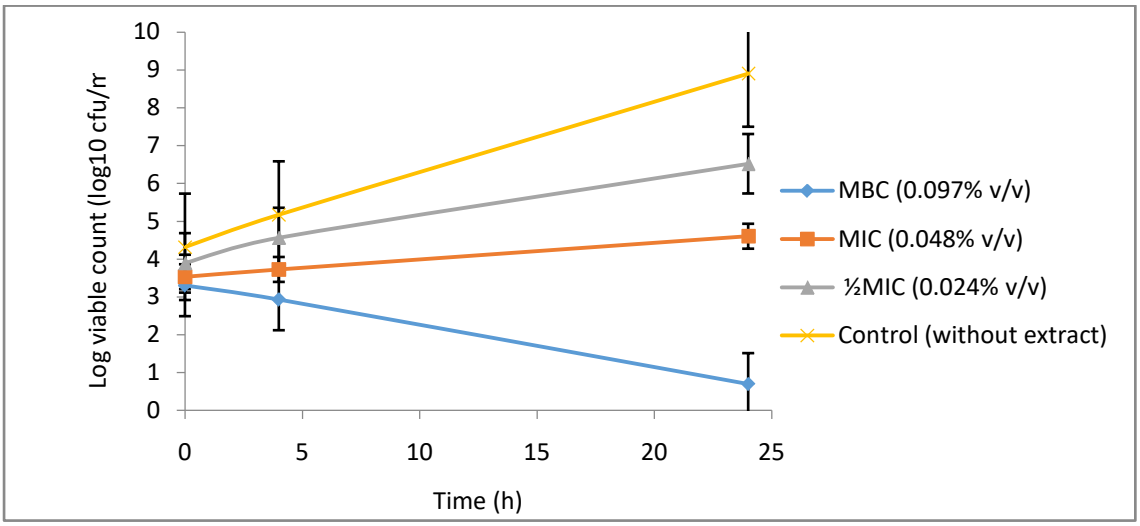

***Figure 3*:** Growth and survival of *B. cereus* (University of Wolverhampton strain 110) in rice cooked with salt containing basil essential oil at the concentrations MBC, MIC, ½ MIC and control after incubation at 30^0C for 4 and 24 hours; N=3.

4. Discussion

Bacillus cereus is a common contaminant found in raw rice with raw husked and unhusked rice containing 2.5×10^1 and 2.5×10^3 cfu/g respectively (Cronin and Wilkinson, 2009). Although inappropriate handling, kitchen hygiene and temperature control are contributing factors, cooked rice is mostly associated with *B. cereus* emetic food poisoning (Tewari and Abdullah, 2014; Organji *et al.*, 2015).

In this study, microbial analysis carried out on rice showed the absence of *Bacillus cereus* in both raw and cooked rice samples. In contrast, Fangio *et al.*, (2010), detected 100% *Bacillus spp.* in unhusked rice and 83% incidence in white rice samples. Studies carried out by Organji *et al.*, (2015), showed 75% of raw rice in Saudi Arabia and Egypt are contaminated with *B. cereus* to a varying degree. Analysis of 178 raw rice samples from retail food stores in the USA showed the presence of *Bacillus spp.* spores in 94 (52.8%) of the samples with 83 of the isolates being identified as *Bacillus cereus* (3.6-460 cfu/g) and 11 as *Bacillus thuringiensis* (3.6-23 cfu/g) (Ankolekar and Labbé, 2009). Furthermore, an exposure assessment study carried out by (Dong, 2012) on Chinese-style cooked rice in Shanghai, China showed that approximately 3.07% of cooked rice contained more than 4 Log cfu/g of *B. cereus*. The absence of *B. cereus* in the rice sample used in this study might be due to processing steps of milling and polishing. This is in accordance to studies carried out by Ankolekar and Labbé, (2009), who determined the level of *B. cereus* on 43 and 89 samples of brown and white rice respectively. The results showed the presence of *B. cereus* in 55.1% (52.9 cfu/g) and 47.2% (30.2 cfu/g) of brown and white rice respectively. Furthermore, the

combined use of 30% fermented ethanol (FE) for 60 minutes and 200bar pressure of Super-Critical carbon dioxide (SC-CO_2) at 44^0C for 30 minutes successfully reduced *B. cereus* in raw rice by 5 Log cfu/g (Kim *et al.*, 2013). According to Food Standards Agency (FSA) (2012), exposing cereals to 1kGy of either gamma rays, electron beams or x-rays is a safe way of killing food poisoning bacteria and extending the shelf life of food. This method is referred to as 'irradiation' and irradiated foods are labelled as 'treated with ionising radiation' or 'irradiated'. However, to achieve 90% reduction of vegetative cells and spores, a dose of 0.17-0.65 kGy and 1.25 - 4kGy respectively is required (Tajkarimi, 2007). Nevertheless, the rice used in this study was not labelled as 'irradiated', thus suggesting that a different method of sterilization might have been used to treat the rice. Another possible reason might be the rice is naturally not contaminated.

In this study, basil essential oil showed antimicrobial activity against the test organisms. Using the agar diffusion method, 100μl of the oil was placed into 6mm well and growth inhibition was seen against all organisms tested (*B. cereus* UW strains 10, 110 and 555) to a varying degree. At concentrations of 12.5%, 25%, 50%, and 100% (v/v), basil oil showed zones of inhibition ranging from 13-40mm. According to Sethi *et al.,* (2013), basil oil showed antimicrobial activity against *B. cereus* with zone of inhibition of 12mm. Using the agar disc diffusion method, zones of inhibition of 16.11mm, 23.58 and 30.56mm against *B. cereus*, *E. coli* and *S. aureus* respectively were observed with basil oil (Moghaddam *et al.,* 2011). Similarly, basil oil showed a zone of inhibition of 13.58mm against *B. cereus* (Semeniuc *et al.,* 2016). According to Chenni *et al.,* (2016), using 6mm discs, antimicrobial activity of basil oil obtained by two different extraction methods ''Solvent-Free Microwave extraction (SFME) and Hydro-Distillation extraction (HDE)'' showed a slight difference in zones of inhibition against organisms tested. *B. subtilis* and *S. aureus* had zones of inhibition of 37mm; 38mm for SFME and 34mm; 33mm for HDE respectively. This might also be the reason why basil oil used in this study has a stronger antimicrobial activity than those of the literatures searched. Another reason might be the methods used in determining the antimicrobial activity.

The MIC results of basil oil in this study was obtained using a doubling dilution series in TSB. For basil oil, both *B. cereus* UW strains 10 and 555 had an MIC value of 0.097% (v/v), while strain 110 has an MIC value of 0.048% (v/v) proving to be the most sensitive. In contrast, *B. cereus* 10 and 110 had an MBC value of 0.97% (v/v) while strain 555 has an MBC value of 0.195% (v/v). Moghaddam *et al.,* (2011), reports that MICs of basil oil against *B. cereus*, *S. aureus* and *E. coli* ranges from 36-18μg/mL, 18μg/mL and 18-9μg/mL

respectively. Basil oil from blue spice and lattuga cultivars showed MIC and MBC values of 0.11μg/mL and 0.57μg/mL; 0.14μg/mL and 0.67μg/mL respectively against *B. cereus* (Beatovic *et al.,* 2015). Similarly, sweet basil has also been shown to have an MIC and MBC values of 50μg/mL and 100μg/mL respectively against *B. cereus.* Comparing studies on the antimicrobial activities of basil oil is difficult or rather impossible due to many differences including methods of evaluating antimicrobial properties and also differences in compositions or herbal contents of basil from different geographical regions. However, basil oil proves to be effective against all the 3 strains of *B. cereus* tested in this study.

Despite being of the same species, the strains of *B. cereus* used in this study showed different susceptibility to essential oils. These results are in agreement to those of (Nazzaro *et al.,* 2013), who observed that 2 strains of *B. cereus* (DSM 4313 and DSM 4312) behaved differently when exposed to the same extract. Possibly, different strains of *B. cereus* have different cell structure which may perhaps have effect on their susceptibility to plant extracts. Hence, this results suggests further research on the mode of action of plant essential oils and their components on multiple strains and species of microorganisms.

In this study, the growth and survival of *B. cereus* (UW strain 110) in cooked rice samples containing basil oil at concentrations of 0.097% v/v (MBC), 0.048%v/v (MIC) and 0.024% v/v (½ MIC) after incubation at 30^0C for 24 hours were determined. The results showed a significant reduction in bacterial count from 3.5 $\log_{10}$ cfu/ml to 1.1 $\log_{10}$ cfu/ml at MBC concentration. Even though at the MIC and ½ MIC concentrations *B. cereus* count increased from 4.1 $\log_{10}$ cfu/ml to 4.8 $\log_{10}$ cfu/ml and 4.6 $\log_{10}$ cfu/ml to 6.6 $\log_{10}$ cfu/ml respectively, this was better than the control without basil oil which showed an increase from 4.6 $\log_{10}$ cfu/ml to 9.2 $\log_{10}$ cfu/ml. In a similar study, essential oil from basil proved to be effective against *B. cereus* in 6 types of rice after incubation for 2 hours at 37^0C at concentrations of 40μl and 80 μl (Budkha and Khan, 2010). The survival of *Salmonella enterica* serotype *enteritidis* (D) ATCC 13076 was inhibited by addition of basil oil at concentrations of 1%, 2.5% and 5% (v/v) during different stages of processing. The greatest reduction of 0.76 log cfu/g was seen during cooling process at 5% (v/v) concentration (Stojiljković *et al.,* 2015).

Results of the antimicrobial activities of basil oil on rice samples containing 0.2% (w/v) concentration of salt appeared to be very similar to those without salt. This is in contrast to the findings of Rivera *et al.,* (2015), who reports that increasing salt content in food matrices can enhance the antimicrobial activity of essential oils. Hence, this study demonstrates that basil oils antimicrobial activity against *Bacillus* was not enhanced by salt but this may be due to the salt tolerant nature of *B. cereus.*

Basil essential oil at concentrations of 0.0625%, and 0.125% and 0.25% reduced the growth rate of *S. aureus* for 12 days at a storage temperature of 4^0C in beef burger with optimum organoleptic concentration of 0.125% (Sharafati *et al.*, 2015). The growth of *S. enteritidis* in fermented pork sausage was reduced from 5 to 2 log cfu g^{-1} by 0.005% concentration of basil essential oil after 3 days of storage at 4^0C. While at concentrations of 0.01% and 0.015%, growth was reduced to 1 log cfu g^{-1} but sensory evaluation suggested that addition of 0.01% but not of 0.015% would be acceptable to consumers (Rattanachaikunsopon and Phumkhachorn, 2010).

Moreover, essential oil from basil has been shown to exhibit bactericidal properties in rice-based foods at different concentrations of 10, 20, 40 and 80μl at temperatures of 25 and 30^0C. Optimum bactericidal activity was achieved with 80μl amount of oil (Budka and Khan, 2010).

5. Conclusion

Bacillus cereus is a common contaminant found in rice causing the emetic type of food poisoning which is often associated with starchy foods. Chemical preservatives like benzoates are used as antimicrobials but consumer concern of the toxicity and long-term carcinogenic effect of these chemicals has led to the demand of minimally processed, chemical free and high quality foods with extended shelf life. This study showed that basil essential oil exhibits a promising antimicrobial effect against *Bacillus cereus* in cooked rice samples with and without salt. This means the use of basil oil can replace the use of salt as a preservative.

In conclusion, basil oil can be a potential candidate to be used as a natural alternative for further application in food preservation to inhibit the growth of *Bacillus* and subsequently increase the shelf life of rice and other starchy foods. However, the application of this essential oil to control pathogenic and spoilage bacteria in foods needs further evaluation. This includes the concentration required for activity, organoleptic impact, chemical composition of food and interference to the antimicrobial action. Also, the characteristics of food spoilage microorganisms needs to be considered.

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