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Effects of Environmental factors on the Growth and Proliferation of Yeasts

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Abstract

Yeast is one of the organisms that grow and survive in diverse conditions. The influence of environmental factors on its growth and proliferation were evaluated. The yeast used for the study was isolated from honey using Sabouraud Dextrose Agar and pour plate methods. The effects of temperature, pH and ultraviolet radiation on the growth of *Rhodotorula*, *Debaryomyces*, *Zygosaccharomyces* and *Candida* species were determined using standard methods. The growths of all the yeast isolates were greatly affected at 80°C. *Rhodotorulla* species showed least growth at 50°C, but optimal growth was recorded at 30°C after 96 h of incubation. A similar trend was observed in *Debaromyces* species. *Zygosaccharomyces* species recorded high reduction in growth at 60°C after 96 h of incubation, though there were no significant differences in the growths of the isolates at 60°C and 80°C. *Candida* species recorded the least effect of temperature at 30°C and highest at 60°C and 80°C after 96 h of incubation. The results of the effect of pH on growth of the yeasts showed that *Rhodotorula* and *Zygosaccharomyces* had optimum growth at pH of 5 and 4 respectively. All the isolates showed increase in growth with increase in incubation time. Exposure of the isolates to UV-rays negatively affected the growths of the isolates. The growths of the yeasts decreased with increase in exposure time. This study showed that yeast can survive adverse temperature and pH and can as well survive UV-ray exposure.

Keywords: Yeast, Temperature, pH, UV-ray.
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Introduction

Microorganisms have a relatively large tolerance range for changes in environmental conditions. Under the right conditions, microorganisms thrive very well. The adverse effects may cause the inhibition of cell growth, damage, or lead to the death of the microorganism (El˙zbieta, 2020). Several environmental factors can influence proliferation of yeasts. Temperature changes are an important factor that increase or decrease the life of yeast. Some factors such as sun-rays and pressure exhibit less effect and they are less studied (Gabor and Carlos, 2006).

The temperature range for proliferation of yeasts varies with species; it is usually between 20°C and 30°C (Jaruwan and Jirajin, 2009). Findings from a study involving about 600 strains of different species of yeasts showed that the growth limit was between 24°C and 50°C (Vidal-Leira *et al.,* 1979). At 37°C, only few species such as *Candida albicans* and a few opportunistic yeasts can grow. Most strains of yeast that are commonly used in industries can grow at 37°C.

The limiting temperature can be below 0°C for yeast that tolerate very low temperature. A

yeast can be referred to as psychrotrophic if it grows at temperatures between 0 and 25°C and mesophilic if it grows at 5–10°C (Vidal-Leira *et al*., 1979). Influence of low water activity, low pH level and antimicrobial components can minimize the growth temperature range (Banat and Marchant, 1995). The growth and development of microorganisms is stimulated by external stimuli, i.e., environmental factors. These factors determine the possibility of the growth of specific microorganisms (Godd and Dyer, 2017). The main growth factors are temperature, humidity, pH level, concentration of hydrogen ions in the environment, oxido-reduction, water activity in the environment, and hydrostatic pressure (Piontek and Lechow, 2013).

Yeasts do not require light to grow; hence, light is not vital for their proliferation. Few researchers discovered impact of light on them, but they refer it to the possible effect of UV-rays. This may inform the abundance of coloured species such as *Cryptococcus* and *Rhodotorula* on the leaves. A study by Andrews *et al*. (1980) suggested that leaf position within apple tree canopy substantially influence the resistant population. Yeasts are commonly seen in salt water habitats but studies have shown its presence in sediments and benthic region from deep sea of 2,000–6,500m (Nagahama *et al*., 2001; Palhono*et al*., 2004). Further data from yeast cells baro-resistance was collected on the studies which identified possible application of hydrostatic pressure at high level to preserve food in industries (Smelt, 1998). Pressure at high level has a significant effect on cell composition of yeasts (Palhano *et al*., 2004). When yeasts were made vulnerable to slight shock, it makes yeast unsusceptible to pressure. Thus, hints at the activities of general mechanisms of yeast cells response to stress that protecting them against other stress factors. The objective of this study was to evaluate the effect of different environmental factors on the growth or proliferation of yeast.

Materials and Method

Sample collection

Honey samples were collected in screw-capped sterile containers from Ogige market in Nsukka, Enugu State, Nigeria. The samples were randomly collected from the sellers in Ogige market.

Preparation of phosphate buffer

Phosphate buffer (0.1 M) solution was prepared by dissolving the respective quantities (1.46 g and 1.24 g) of Na_2HPO_4 and NaH_2PO_4 in a volume (100 ml) of distilled water. The mixture was reduced to pH 4.0 using 0.1 M NaOH and Phosphoric acid. Distilled water was then used to make up to 100 ml volume. Exact method was used to prepare 0.1 M phosphate buffer solutions of pH 5.0, 6.0, 7.0, 8.0 and 9.0.

Preparation of Phosphate-buffered Sabouraud Dextrose Broth

Sabouraud dextrose broth was prepared according to manufacturer's specifications. A volume of 95 ml of the broth was added to 5 ml of 0.1 molar phosphate buffer prepared above to give a volume of 100 ml phosphate-buffered Sabouraud dextrose broth. Adjustments were made to ensure that the buffered Sabouraud dextrose broth was at the required pH. This was followed by sterilization and inoculation with the test organism. Growths of the isolates were determined at 24 h intervals with a spectrophotometer at 600 nm.

Isolation of yeasts from the honey sample

The honey samples were serially diluted (10-fold) in sterile distilled water and plated on Sabouraud Dextrose Agar (SDA) using spread plate method. Plates containing the samples were incubated at 30°C for 96 h. Distinct colonies were randomly picked and sub-cultured repeatedly. After successful purification on SDA the isolates were kept on slant at 4°C.

Preparation of inocula

The isolates were collected from the stock culture and inoculated in Sabouraud broth and incubated for 48 h. The yeast cells were harvested and re-suspended in distilled water to ensure equal cell population of each of the yeast cells. The optical density was adjusted to be the same value using a spectrophotometer (Jenway 6405, UK) at 660nm.

Determination of the effect of temperature on the growth of the isolates

The effect of temperature on the growth of the isolates were determined by preparing the growth medium (Sabouraud dextrose broth) and dispensed in 100 ml portions into different 250 ml conical flasks and sterilized at 121^{o}C for 15 min. The medium was inoculated with 1.0 ml of the test organism and incubated at different temperatures (20, 30, 40, 50, 60 and 80^{o}C) for 96 h. At 24 h intervals, the growths of the isolates were determined using spectrophotometer (Jenway 6405, UK) at 600 nm.

Determination of the effect of pH on the growth of the isolate

The pH effects on the growth of the isolates were evaluated using phosphate-buffered sabouraud dextrose broth.

Effect of Ultraviolet Radiation on the Growth of the Isolates

The effects of ultraviolent radiation on the growth of the isolates were determined using ultraviolet box at a wavelength of 260 nm. A portion (0.1ml) of the isolate was aseptically inoculated into SDA plate using spread plate method. Each plate was exposed at different times (5, 10, 20, 30, 40 and 60/min) using a UV box and the plates were incubated at room temperature for 48 h. The growth of the isolates by plate count was compared with the plates that were not exposed to UV, which served as control.

Data analysis

The data obtained were analysed using one-way and two-way analysis of variance (ANOVA) at 95% confidence interval for statistical significance.

Results

Effect of temperature on the growth of the isolates

The effect of temperature on the growths of the isolates were evaluated and represented in Figs. 1 to 4. Fig.1 showed the effect of temperature on the growth of *Rhodotorula* sp. where growth decreased with increase in temperature. The isolate showed optimum growth at 30^{o}C. The growth of *Debaryomyces* sp. at different temperatures is presented in Fig. 2. The organism showed optimum growth between 20 and 40^{o}C. The growth of the isolate at different temperature was not significant ($P \leq 0.05$). The results of the growth of *Zygosaccharomyces* sp. at different degrees of temperatures were represented in Fig. 3. The isolate showed optimum growth at 30^{o}C and least growth at 80^{o}C. There was no significant difference ($P \leq 0.05$) in the growth of the isolates at different temperatures and time. Fig. 4 showed the growth of *Candida* sp. at different temperatures. This isolate showed optimum growth between 20 and 40^{o}C.

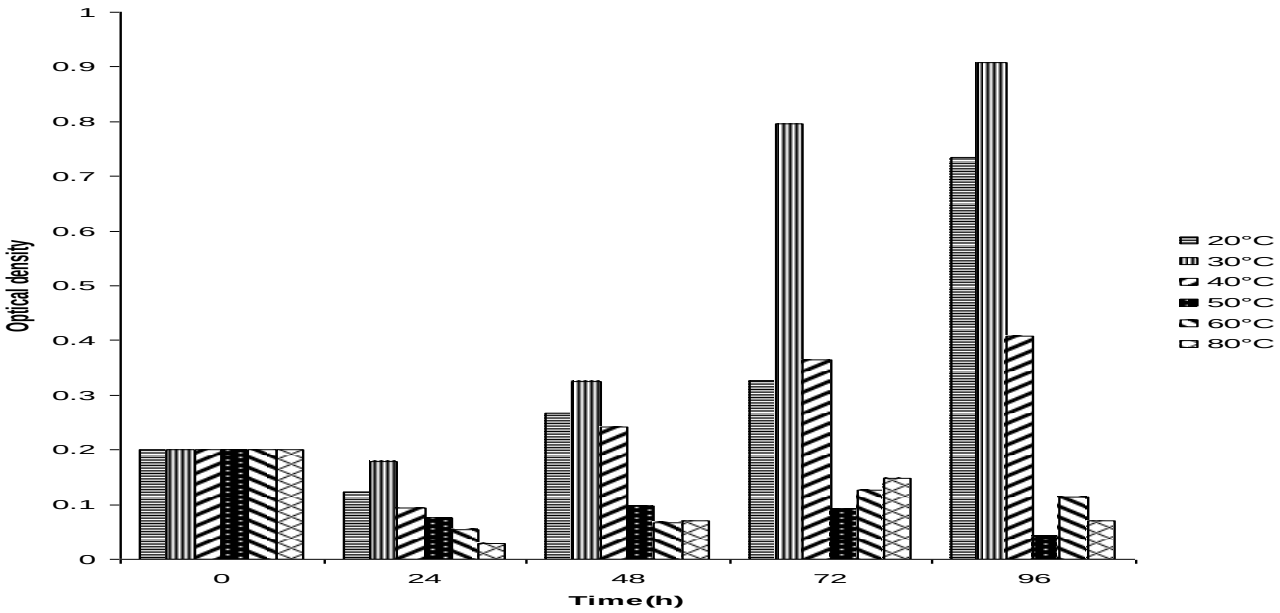

Fig.1: Effect of temperature on the growth of *Rhodotorula* species.

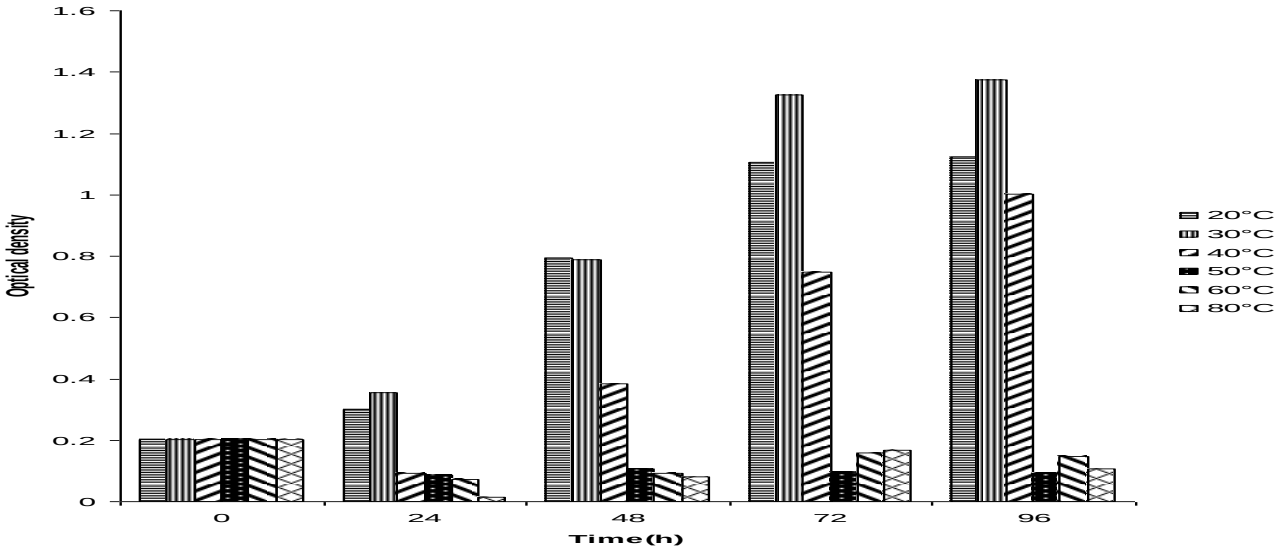

Fig.2: Effect of temperature on the growth of *Debaryomyces* species.

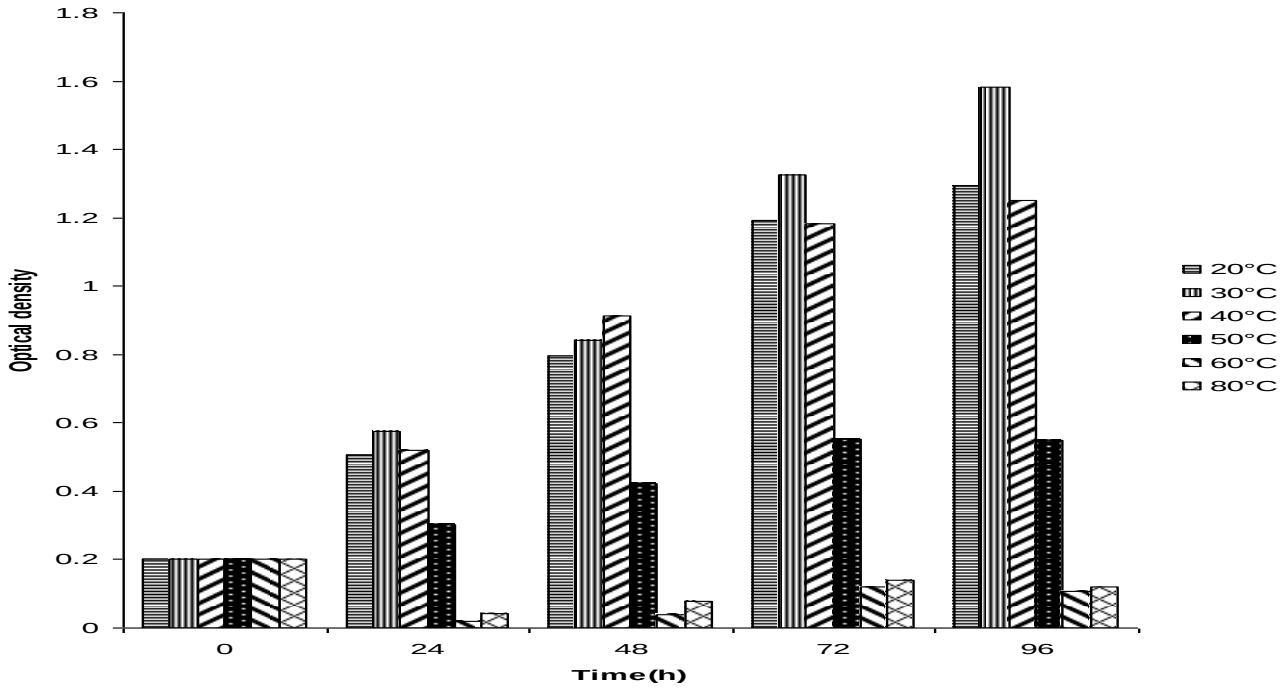

Fig.3: Effect of temperature on the growth of *Zygosaccharomyces* species.

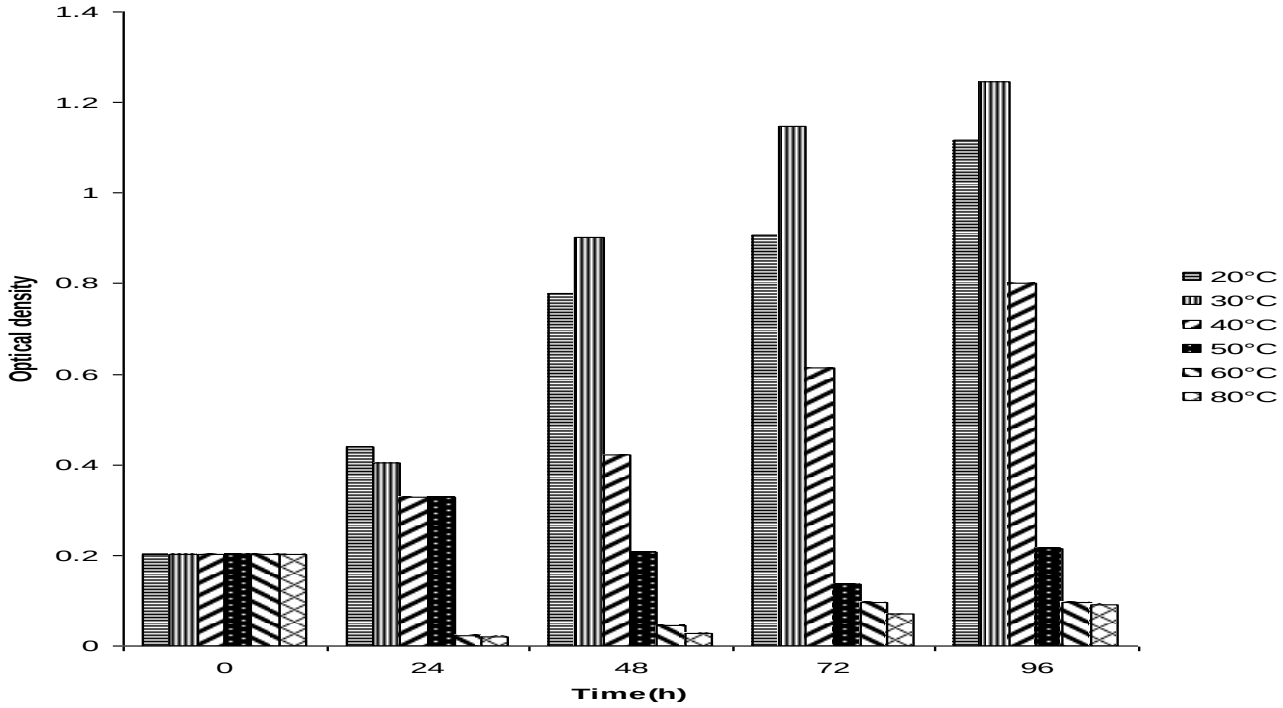

Fig.4: Effect of temperature on the growth of *Candida* species.

Effect of pH on the Growth of the Isolates

The effects of pH on the growth of the isolates were presented in Figs 5 to 8. The results of the growth of *Rhodotorula* sp. at different pH values showed optimum growth at pH of 5. However, at pH 4, the growth of the isolate declined while the least growth was recorded at pH of 9 (Fig. 5). *Debaryomyces* sp. had optimum growth at pH of 5 and least at pH of 9 (Fig. 6). Significant difference was not observed in the growth of the isolates at different pH. *Zygosaccharomyces* sp. showed optimum growth at pH of 4 (Fig. 7). There was no significant difference in the growth of the isolate at pH of 4 and 5 and the isolate showed the least growth at pH of 9. *Candida* sp. had optimum growth at pH 5 (Fig. 8). Significant difference was not noticed in the growth of the isolate at difference pH

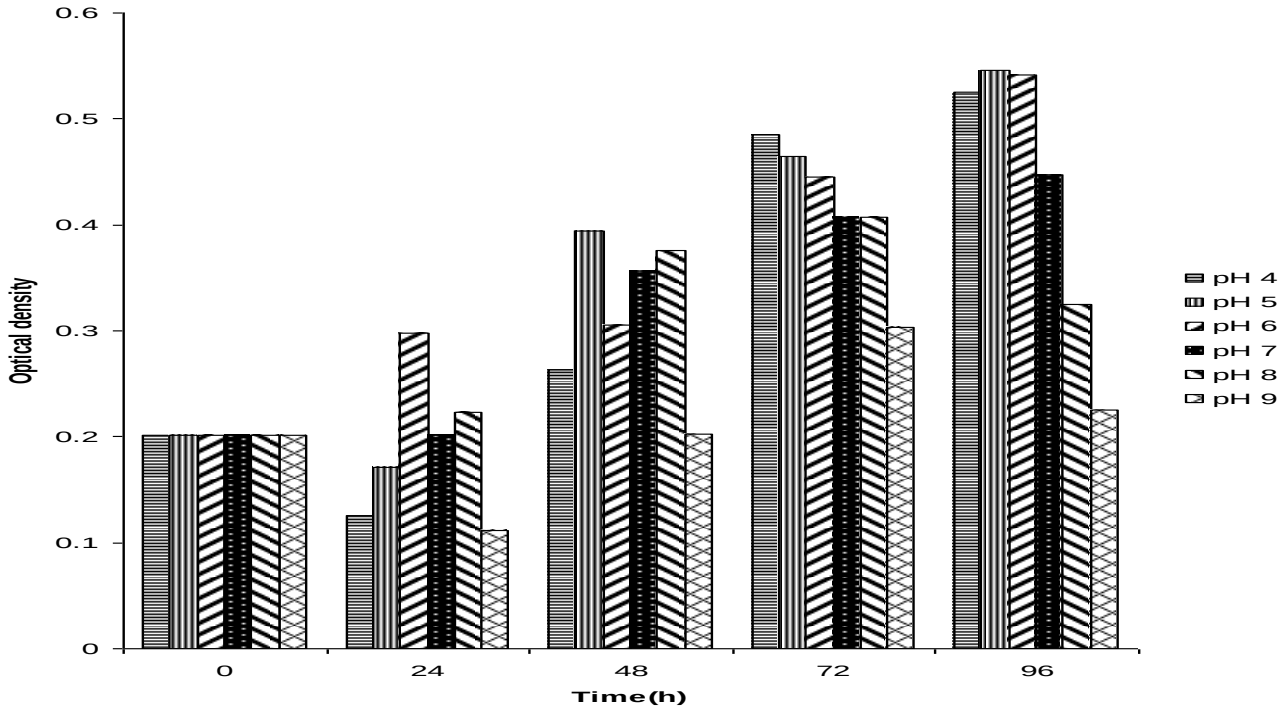

Fig.5: Effect of pH on the growth of *Rhodotorula* sp.

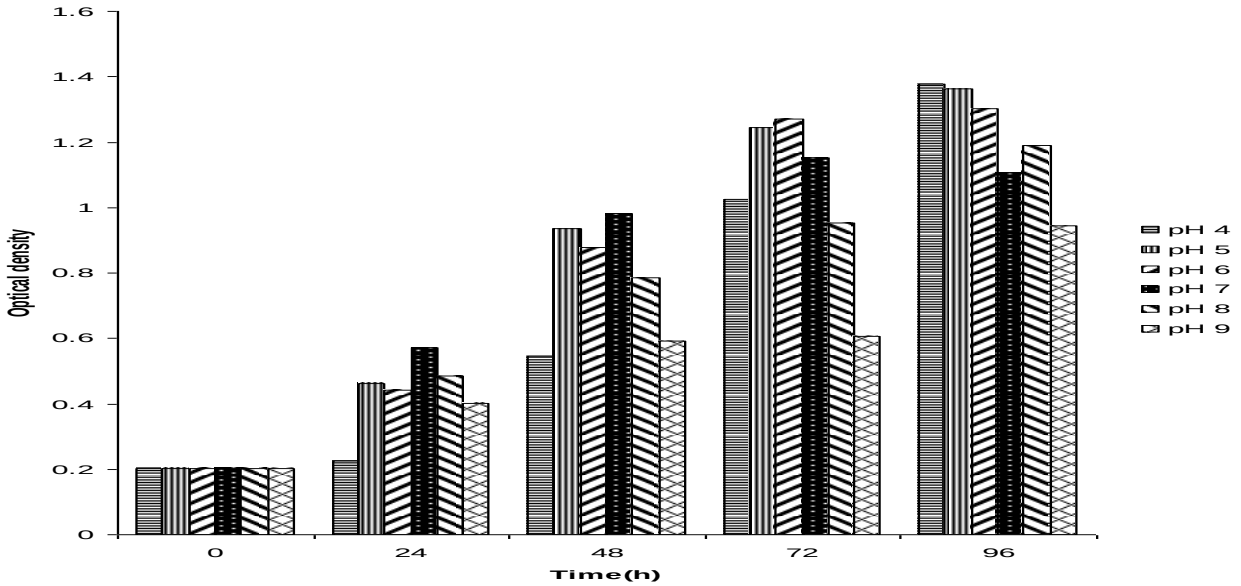

Fig.6: Effect of pH on the growth of *Debaryomyces* sp.

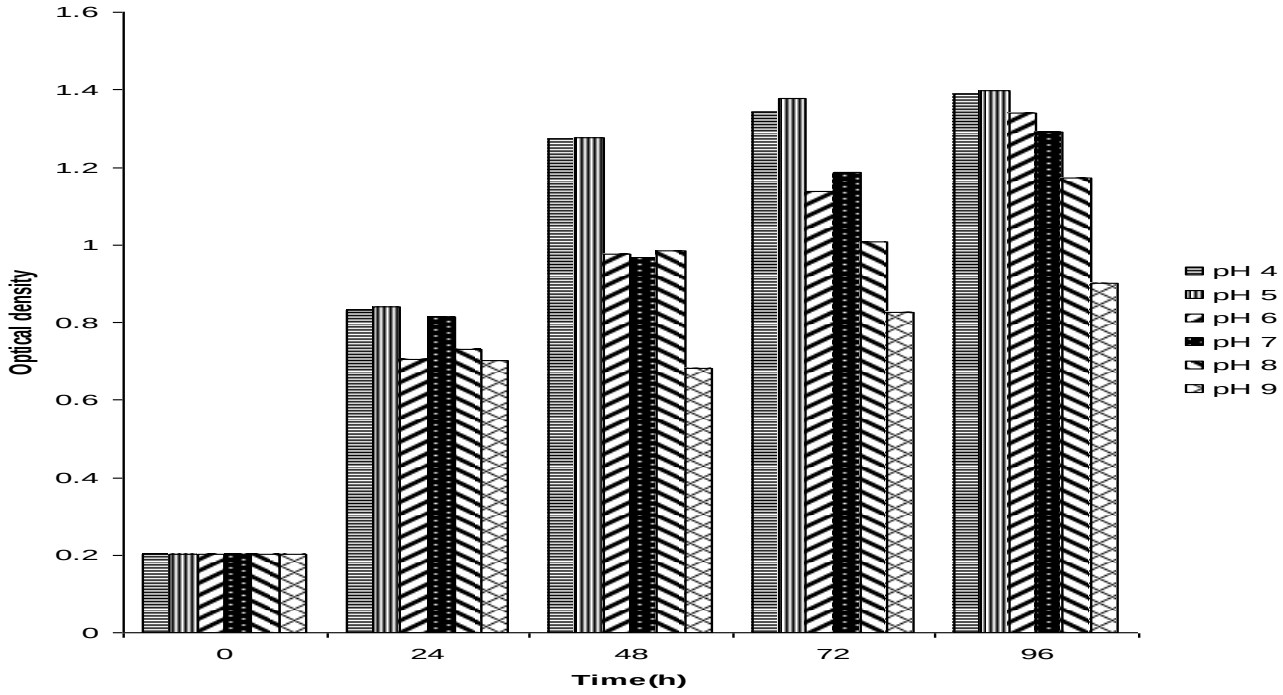

Fig.7: Effect of pH on the growth of *Zygosaccharomyces* sp.

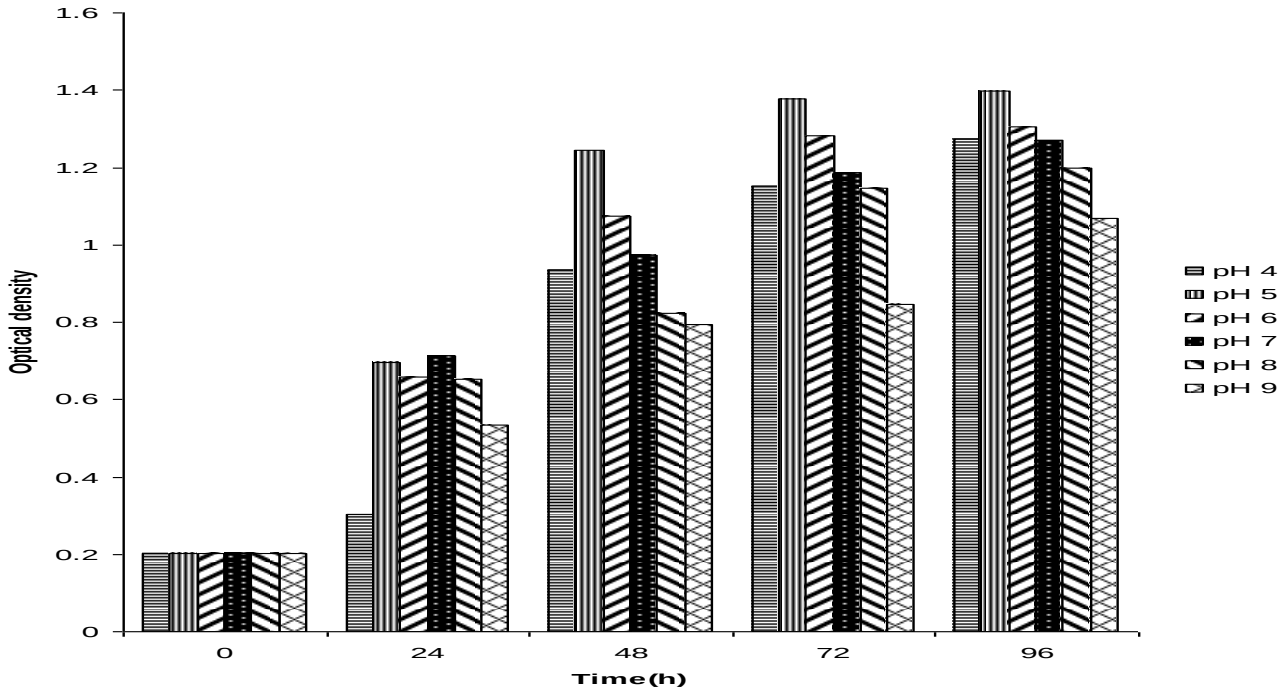

Fig.8: Effect of pH on the growth of *Candida* sp.

Effect of Ultra Violet (UV) Radiation on Growth of the Isolates

The results of UV radiation on the growth of the isolates were presented in Fig. 9 to 12. All the isolates showed optimum growth when they were not exposed to UV radiation. The effect of UV radiation on the growth of *Zygosaccharomyces* sp. is showed in Fig. 9. The organism recorded a minimal effect of UV radiation at 5 mins of exposure. The least observable colonies were obtained at 60 mins of incubation. The effect of uv on the growth of *Rhodotorula* sp. is presented in Fig.10. There was a decline in growth as the time of the exposure increases. The growth was almost inhibited at 60 mins of exposure. The result of the effect of uv on the growth of *Debaryomyces* sp. is presented in Fig.11. The growth of the isolates decreased with increase in exposure time. The isolate also showed a very low growth at 60 mins of exposure. Fig. 12 showed the cell growth as a result of UV radiation on the *Candida* sp. The growth of the isolate was apparently high when they were not exposure on UV radiation and decreased with increase in the time of exposure to UV radiation.

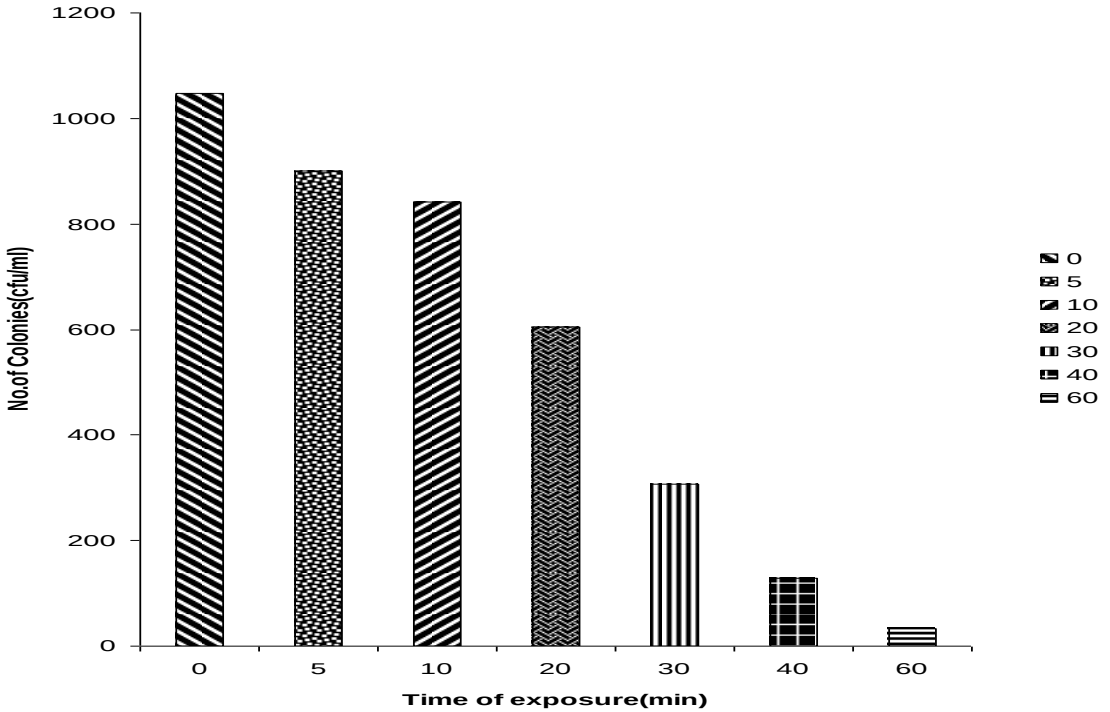

Fig: 9: Effect of UV radiation on the growth of *Zygosaccharomyces* sp.

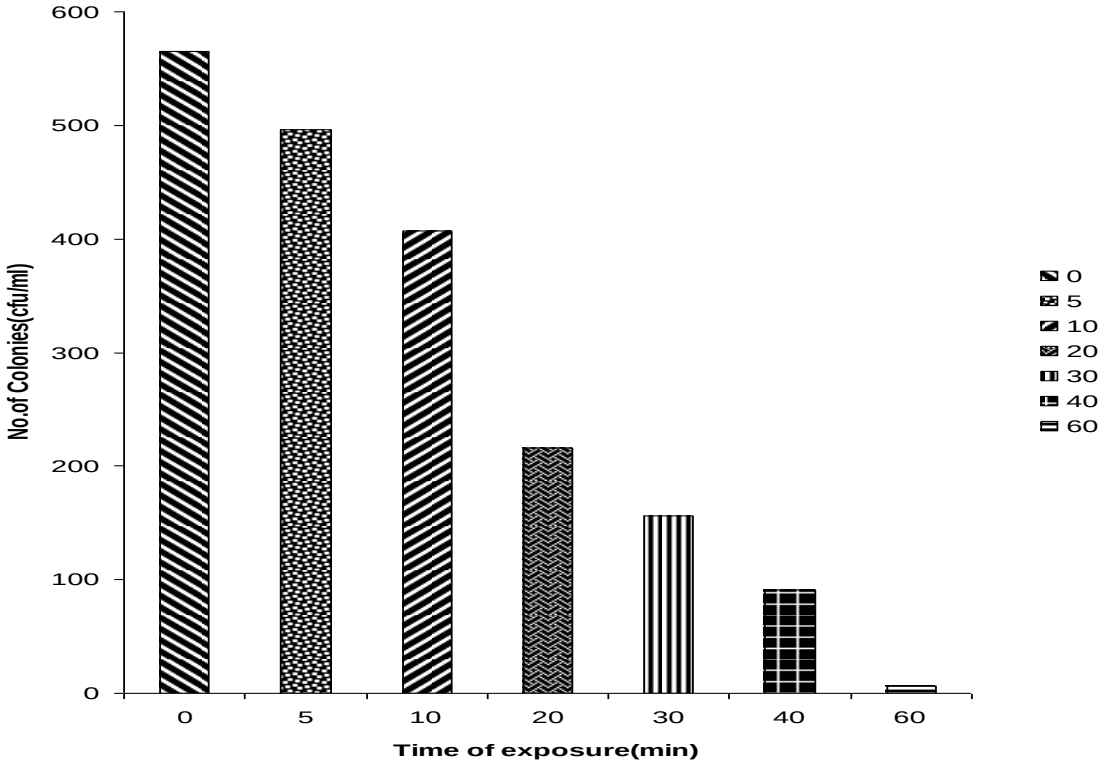

Fig: 10: Effect of UV radiation on the growth of *Rhodotorula* sp.

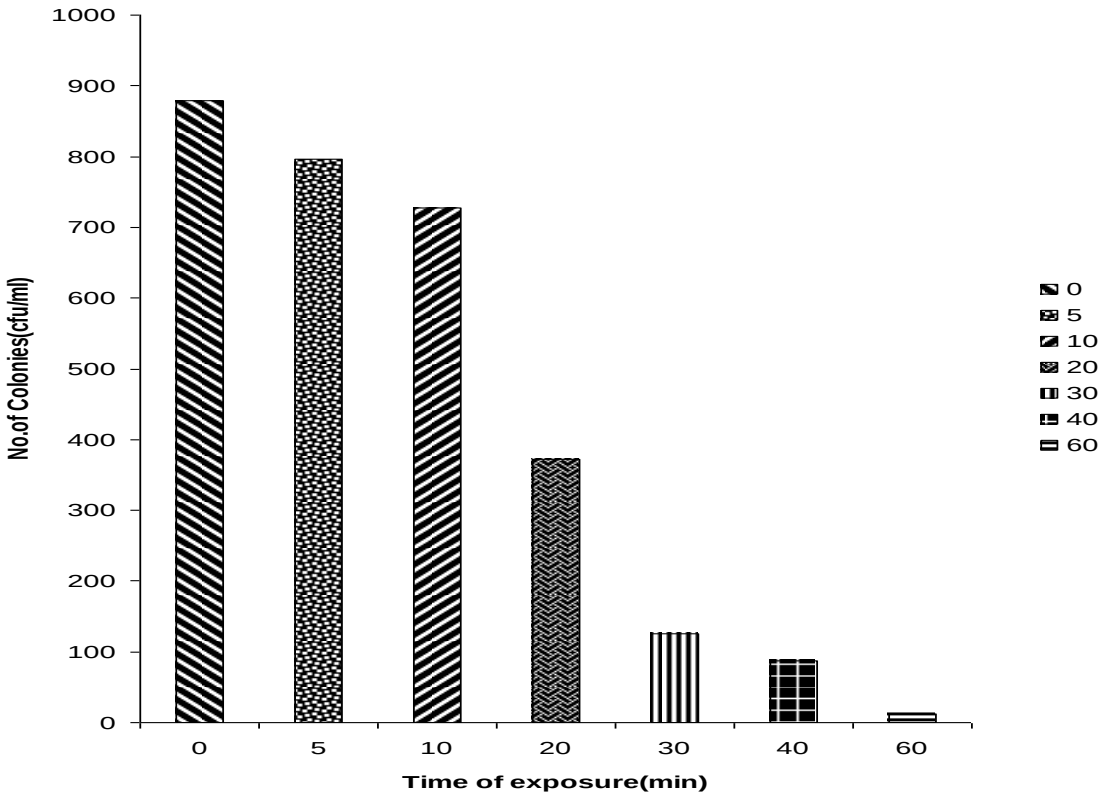

Fig: 11: Effect of UV radiation on the growth of *Debaryomyces* sp.

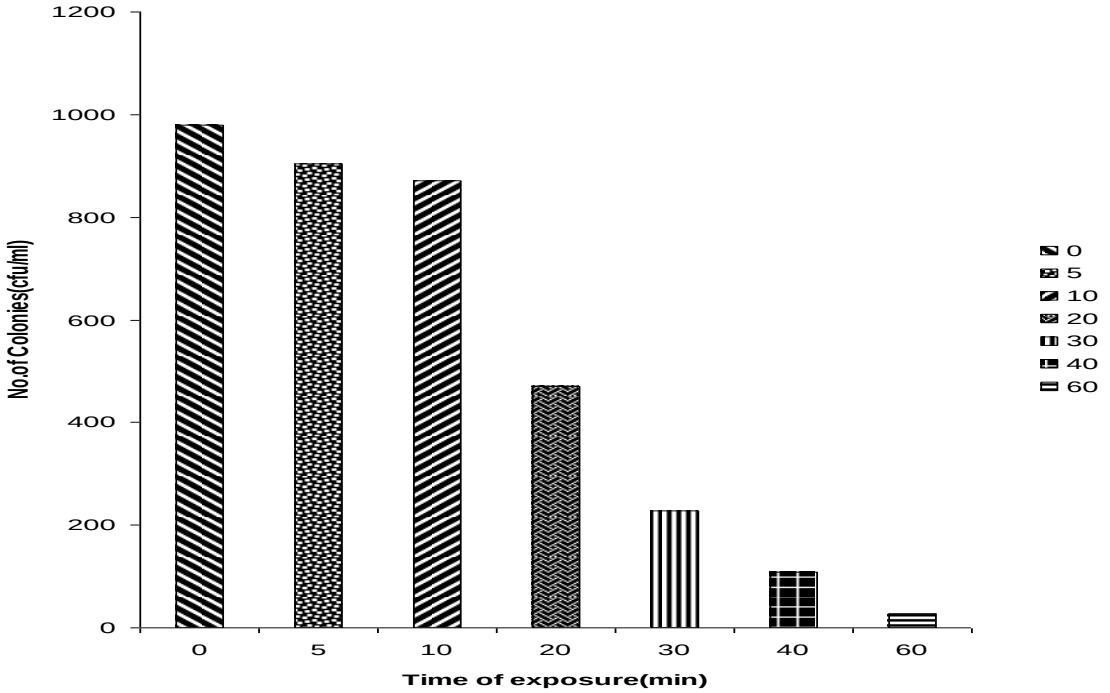

Fig. 12: Effect of UV radiation on the growth of *Candida* sp.

Discussion

This study observed that environmental factors have a little or no signification effect on the growth and proliferation of the yeast cells. Thus, it is in line with the findings of other researchers on the effected of certain environmental factors such as temperature, pH, oxidative stress and solute stress on the growth and proliferation of yeast (Sui *et al*., 2015). These factors help to determine the survival of the yeast cells present in the environments, as they bring changes in the environment that can favour or discourage the growth of the yeast cells (Spadaro and Droby, 2016). *Rhodotorula*, *Debaryomyces*, *Zygosaccharomyces* and *Candida* species were discovered to have different effects or changes in growth at different environmental parameters such as pH, temperature, and UV radiation. This changes maybe as a result of alteration in the environmental composition which may result to change in nutrient composition of the environment that may or may not enhance yeast stress or growth tolerance (Wang *et al*., 2018). These changes can be physical, chemical or biological. The changes may need specific response mechanism so as to protect and make the cell to survive the new conditions (Hohman and Mager, 2003).

Rhodotorula species showed maximum growth at 30^{o}C and least growth at 80^{o}C. There was no significant difference (P ≤0.05) in the growth of *Debaryomyces* sp. at 50, 60, and 80^{o}C respectively, notably high growth rate was observed at 30^{o}C. *Zygosaccharomyces* sp. had the highest growth at 30^{o}C while the *Candida sp* showed the highest growth at 20^{o}C. There was no significant difference (P≤0.05) in the growth of the isolates at different temperatures and time. The decrease in the growth of the organisms at high temperature may be due to the decrease in enzyme activity which results from the denaturation of the enzymes as a result of the high temperature. Manet (2006) reported that enzyme activity is optimized at certain temperature range, beyond which the enzymes undergo denaturation and his findings is in agreement with the present observation of this study.

The responses of the yeast cells to different pH as evaluated in this study showed the organisms had similar pH range for their growth, though some had higher pH range than the other, but the differences are not significant (P ≤ 0.05). *Rhodotorula* species gave the highest growth at a pH of 5.0 when grown for 96 h in incubation. The growth of *Debaryomyces* was also influenced by pH, and least growth was noticed at pH of 9.0. The growth of *Zygosaccharomyces* sp was also observed best at the same pH value with *Debaryomyces* species. The isolate was able to grow at different PH ranges. *Candida* showed a progressive growth at different pH. The highest growth was showed at pH of 4 and 5 while the least growth was obtained at pH of 9. There was no significant difference in the growth of the isolates at different pH between 0 and 24 h of incubation. These results agreed with the work of Jaruwan and Jirajin (2009) which reported that all yeasts grow best at pH 3.0 - 7.0. They reported that varying pH was shown to have a positive effect on growth. Martina *et al.* (2007) also demonstrated that pH affects growth, membrane fluidity and lipid concentration of yeasts and that low pH (pH 4) caused a significant decrease in membrane fluidity. This change in membrane fluidity may indicate that at low pH, cells are struggling to survive

The growth response of the osmophilic yeasts to ultraviolet (UV) radiation was also studied at different exposure times. The growth of the isolates decreased with increase in the time of exposure which was more pronounced in some species such as *Debaryomyces* sp*.* All the isolates showed high growth without exposure and least growth at 60 mins exposure. This result agreed with the work of Laila (2004), which reported a decrease in the number of colonies produced by yeasts with increase in the time of exposure to ultraviolent radiation. Caitlin (2006) also reported that the continuous exposure of yeast to UV radiation leads to death of the cell.

Conclusion

The data from this current study have extensively shown the effects of environmental factors on the growth and proliferation of yeasts (*Rhodotorula*, *Debaryomyces, Candidia* and *Zygosaccharomyces* species*)* isolated from honey samples from Enugu State, Nigeria. Their ability to adapt to environmental factors or conditions has been examined with different

parameters and the results proved that the yeast cells growths reduced with the increased in temperature, pH level and time of exposure to UV radiation. This study observed that yeast cells grow best at the temperature range of (20^{o}C to 40^{o}C), pH range of (4.0 to 5.0) and UV radiation exposure time had a great effect on their growth. Thus, environment factors play a very important role in the growth and proliferation of the yeast cells. Therefore, it is important to consider environmental factors since it can enhance the severity of the yeast cells in the environment.

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