FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 8 No. 6, December, (Special Issue) 2024, pp 581 - 586
DOI: https://doi.org/10.33003/fjs-2024-0806-3130

A COMPARATIVE ANALYSIS OF THE ANTIFUNGAL EFFECTS OF YEAST AND BACILLUS SUBTILIS IN THE CONTROL OF BLUE EYE MOLD DISEASE OF MAIZE AND RICE

***[1]Nnebechukwu, Ijeoma Adaku, [1]Rwang Nerat Yusufu,**
[2]Ikechi-Nwogu, Chinyerum Gloria and [3]Ibrahim Omeiza Abdullateef

[1]Department of Plant Science and Biotechnology, Faculty of Natural Sciences, University of Jos
[2]Department of Plant Science and Biotechnology, Faculty of Science, University of Port-Harcourt
[3]Department of Microbiology, Faculty of Science, Federal University Lokoja

*Corresponding authors' email: nnebechukwui@gmail.com Phone: +2347038632042

ABSTRACT

Blue eye mold disease cause significant post harvest losses of grains in storage. The need to control the plant disease with the use of bio friendly agents is of paramount importance. This research assessed the antifungal effect of *Bacillus subtilis and* Yeast (*Saccharomyces cerevisiae)* in the control of blue eye mold disease that affects maize and Rice grains in storage. The diseased grains were gotten from two storage barns which were Bukuru and Katako market storage barns of Jos Metropolis and the ability of these organisms to inhibit the growth of the pathogenic fungi (*Aspergillus glaucus*) found in Maize and Rice grain. The research work was carried out at African Center for Phyto medicine and Research University of Jos, Plateau State. The pathogenic organism was isolated by culturing it on the SDA Media in an aseptic condition. Several other subcultures were carried out in order to get the pure isolates of the fungi. Organisms which served as biocontrol were gotten from National Veterinary Research Institute Vom (NVRI), these organisms were incubated on the same plate with the pathogenic fungi. For *Bacillus subtilis*, it was incubated at 25°C while that of the Yeast was incubated at 30°C. Results showed that *Bacillus subtilis* inhibited at 59.40% while Yeast inhibited at 95.84%in maize grains from Bukuru while *Bacillus subtilis* inhibited at 64.80% and yeast at 69.70%. For Katako market *B. subtilis* inhibited at 57.48% while yeast inhibited at 64.84% for maize grains while for Rice grains *B. subtilis* inhibited at 48.93% and Yeast inhibited at 54.27%. Comparing both organisms that served as Biocontrol agents, Yeast is seen to be the better inhibitor compared to *Bacillus subtilis*. Both organisms are seen to have antifungal effects and perform antagonistic activity against *Aspergillus glaucus* therefore bio fungicides can be made out of these organisms in order to reduce the spread of fungal diseases in stored grains. Further research should be carried out in order to authenticate the effects of these strains produced by the organisms.

Keywords: Yeast, *Bacillus subtilis*, Control, Blue Eye Mold Disease, Maize, Rice

INTRODUCTION

Blue eye mold is one of the most prevalent post-harvest diseases that affect grains and other crops. This condition is caused by the growth of fungal species such as *Aspergillus glaucus* and *Penicillium* spp., which thrive under high humidity and poor storage conditions (Magan & Aldred, 2007). These fungi not only degrade the quality of grains but also pose significant health risks due to the production of mycotoxins, which are toxic secondary metabolites (Miller, 1995). The presence of blue eye mold is strongly associated with improper storage practices. According to Bočarov-Stančić and Miladinović (2015), factors such as high moisture content, inadequate ventilation, and delayed drying contribute to fungal colonization. Grains with moisture levels exceeding 14% are particularly susceptible, emphasizing the need for rigorous post-harvest management (Hell & Mutegi, 2011). Fungal contamination of stored grains like maize (*Zea mays*) and rice (*Oryza sativa*) has severe implications for global food security. Maize and rice are staple foods consumed by millions worldwide, and their contamination during storage can result in significant economic losses and food shortages (Food and Agriculture Organization of the United Nations, 2019). Furthermore, the rapid growth rate of molds, such as those causing blue eye mold, highlights the critical window of 24–48 hours within which preventative measures must be implemented (Ngugi & Scherm, 2006). The impact of fungal contamination extends beyond food supply to health and safety.

Mycotoxins produced by species like *Aspergillus* and *Penicillium* are linked to acute and chronic health conditions, including respiratory problems, immunosuppression, and carcinogenesis (Pitt & Hocking, 2009). Therefore, effective control strategies, such as proper drying, use of fungicides, and improved storage infrastructure, are crucial (Magan & Aldred, 2007). In recent years, alternative approaches to manage fungal contamination have gained attention. For instance, the use of natural antifungal agents, such as plant extracts and other biological organisms such a Yeast and *Bacillus subtilis* has shown promise in mitigating post-harvest diseases (Jia *et al*., 2013). Studies have demonstrated the efficacy of botanical extracts in reducing fungal growth and mycotoxin production, offering a sustainable alternative to synthetic fungicides (Bočarov-Stančić & Miladinović, 2015). Overall, understanding the biological and environmental factors that promote blue eye mold is essential for developing effective prevention and management strategies. As highlighted by Hell and Mutegi (2011), integrating traditional and modern storage practices can significantly reduce post-harvest losses and ensure the safety and availability of staple foods.

MATERIALS AND METHODS

A survey was carried out in different Maize and Rice stores within Jos South and Jos North Local government areas in Plateau State. The survey was done in order to check for the occurrence of Blue eye mold disease affecting the storage barns of Maize and Rice. This was carried out using random sampling methods to check out for disease symptoms. The samples with visible symptoms were collected from Bukuru market of Jos South Local government and Katako market of

Jos North Local government using sterile swabs and adhesive tape for culturing and microscopy.

Biocontrol Organisms Sample Collection

Isolated and already identified organisms (*Bacillus subtilis*) and (*Saccharomyces cerevisiae*) were collected from the Microbiology Laboratory of National Veterinary Research Institute Vom (NVRI) and brought to the Laboratory in African Center for Phytomedicine and Research University of Jos, Plateau State for further use.

Preparation of Media

Sabroaud Dextrose Agar was used to culture the disease grains in order to grow the pathogenic fungi. The media was prepared according to the manufacturer's instructions.

Isolation of Pathogenic Fungi

5ml of 80% Gentamycin was poured in sterile plates. 20 ml of Sabroaud Dextrose Agar was added in the sterile plates and allowed to solidify. The diseased grains were then dropped in 1% Sodium Hypo Chloride for 18 seconds. The grains are then carefully rinsed in distilled water. The grains are then inoculated on then solidified Sabroaud medium under aseptic conditions. The inoculated Petri dishes were the incubated at 30°C for 3 to 5 days. The pure fungal isolates were then obtained after two subcultures on Sabroaud Dextrose Agar. The sub culturing was done by taking a small portion of the pathogenic fungi or the fungi mycelia and then streaked on the media using the wire loop. The culture is then incubated at 30°C for 3 to 5 days. Pure culture of the organism was produced and the macroscopic and microscopic features of the fungi were observed (Zhao *et al.,* 2013)

Identification of Pathogenic Fungi

The fungus was identified microscopically using Lacto phenol and a Light Microscope at X10 magnification. While its cultural characteristics were used for the macroscopic identification

Experimental Design and Antifungal Test

The experiment was laid out in a completely randomized design (CRD) with two biocontrol agents and 5 replicates. These biocontrol agents were *Bacillus subtilis* and *Saccharomyces cerevisiae.* The pathogenic fungi and the biocontrol agents were incubated and inoculated on the same plate. SDA (Sabroaud Dextrose Agar) was prepared according to the manufacturer's instructions, 20mls of SDA was poured in a Petri dish and allowed to solidify. A well was bored in the middle of the media on the Petri dish using a borer. The biocontrol agent which is yeast is then inoculated in the well that was bored. The pathogenic fungus (*Aspergillus glaucus*) was then streaked in a square manner on the surface of the agar on the same plate with the biocontrol agent. The plate was then incubated for 5 days at 37°C (Jia *et a*l., 2013).

In vitro antagonistic assay was performed according to the dual culture method on the SDA Medium. *Bacillus subtilis* was disposed at the center of the Petri dish and the fungal strains were streaked around the agar disk in a square manner. The plate is then incubated at 25°C for 5 days.

Zone of Inhibition

The zone of inhibition was measured using the meter rule in cm. The meter rule was placed at the center of the disk. The measurement was taken from the center of the disk to the edge of the area with zero growth (Bauer *et al.,* 1966)

Percentage of Zone of Inhibition

The percentage of the Zone of inhibition was calculated using the formula

$$Percentage\ Change(\%) = \frac{D5-D1}{D1} \times 100$$

Where:

D5 = Length of the Specimen in cm on day 5

D1= Length of the specimen in cm on day 1

RESULTS AND DISCUSSION

Table 1: Descriptive Statistics of the Antifungal Effect of Yeast and *Bacillus Subtillis* from Bukuru Market Jos

Location	Extract	% Mean ± S.D
Bukuru Market	*Bacillus Subtilis* (Rice)	64.79±50.08
	Yeast (Rice)	69.69±21.40
	Bacillus Subtilis (Maize)	59.39±32.33
	Yeast (Maize)	95.84±35.36
	Total	72.43±36.25

The mean difference is significant at P ≤ 0.05

Table 2: Descriptive Statistics of the Antifungal Effect of Yeast and *Bacillus Subtillis* from Katako Market Jos

Location	Extract	% Mean ± S.D
Katako market	*Bacillus Subtilis* (Rice)	48.93±8.30
	Yeast (Rice)	54.25±13.76
	Bacillus Subtilis (Maize)	57.48±21.64
	Yeast (Maize)	64.83±38.58
	Total	56.37±22.39

The mean difference is significant at P ≤ 0.05

Table 1 shows the antifungal activity of various microbial extracts on grains (rice and maize) collected from Bukuru Market, with results represented as the mean percentage inhibition of fungal growth, along with standard deviation (S.D.). The inhibition rates of the microbial extracts vary significantly between different treatments. Yeast extract from maize shows the highest mean inhibition rate (95.84%) with a fairly high standard deviation (35.36%). Despite the variability, this indicates that yeast may be particularly effective against fungal growth on maize. Bacillus Subtilis extracts, both for rice (64.79%) and maize (59.39%), exhibit lower inhibition rates. This suggests that *Bacillus subtilis* might be less effective than yeast at inhibiting fungal growth on both types of grains. The high standard deviations for some of the results, particularly for *Bacillus subtilis (Rice)* (50.08%) and *Yeast (Maize)* (35.36%), indicate substantial variability in the effectiveness of these treatments. This suggests that the microbial extracts' efficacy may be inconsistent, possibly due to environmental factors or differences in the fungal strains present. On the other hand, yeast extract on rice and maize (69.69% ± 21.40%) demonstrates lower variability, indicating a more consistent effect in inhibiting fungal growth. Yeast seems to perform better on maize (95.84%) compared to rice (69.69%), with a significant difference in inhibition rates. *Bacillus Subtilis* has a similar trend: 64.79% inhibition on rice and 59.39% on maize. The differences suggest that *Bacillus subtilis* might have a slight preference for rice, though the inhibition effect is less pronounced than with yeast. The total average inhibition rate for all treatments is 72.43%, which indicates a moderate level of overall effectiveness in preventing fungal growth.

Table 2 presents the antifungal effects of various microbial extracts on rice and maize at Katako Market, with results in terms of percentage inhibition of fungal growth, including standard deviation (S.D.). Yeast treatment in Maize shows the highest mean inhibition rate at 64.83%, followed by Bacillus Subtilis treatment in Maize at 57.48%. This is consistent with the trend observed in the Bukuru Market results, where yeast also exhibited stronger inhibition on maize. Yeast treatment in Rice at 54.25% and Bacillus subtilis treatment in Rice at 48.93% both show moderate effectiveness in inhibiting fungal growth on rice. *Bacillus Subtilis* appears to be slightly less effective than yeast in this case. There is significant variability in the effectiveness of the treatments, especially in Yeast treatment in Maize at 38.58%. The smaller standard deviations for Bacillus subtilis treatment in Rice at 8.30% and Yeast treatment in Rice at 13.76% suggest relatively more consistent performance in inhibiting fungal growth on rice, compared to the variability seen in maize. Maize generally shows higher inhibition rates than Rice, particularly with Yeast. This suggests that the yeast may be more effective at inhibiting fungal growth on maize. Bacillus subtilis seems to have slightly better performance on Maize (57.48%) compared to Rice (48.93%), but the difference is not as pronounced as with yeast. The total average inhibition rate across all extracts is 56.37%, which is lower than the Bukuru Market average of 72.43%.

Yeast appears to be the most effective treatment for inhibiting fungal growth, with the highest mean inhibition rate of 95.84%. Despite the variability, its overall effectiveness makes it a strong candidate for future applications.

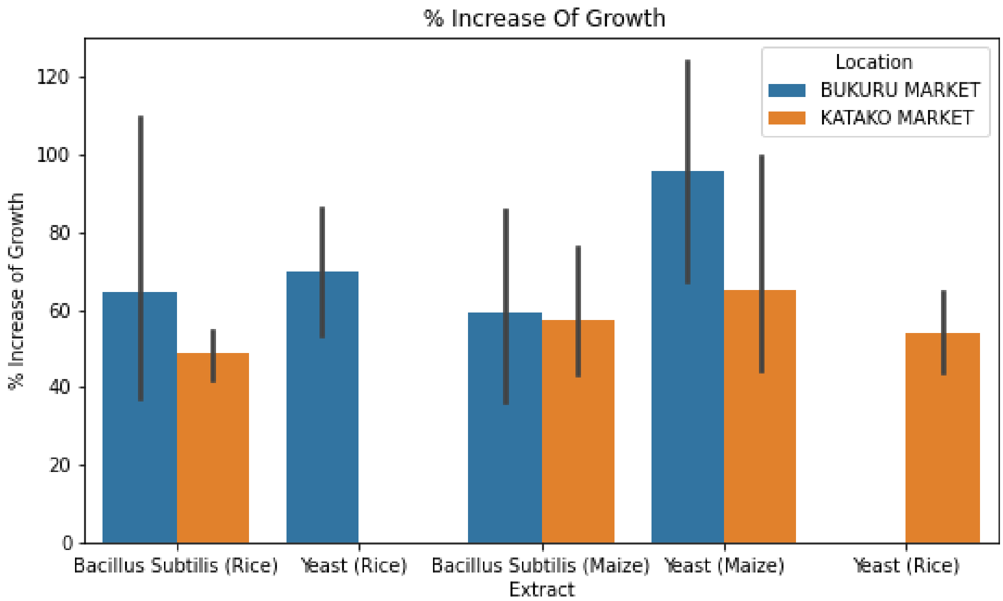

Figure 1: Zone of inhibition result showing the increase in growth of biocontrol agents

Figure 1 shows the comparism of the rate of growth of *Bacillus subtilis* and Yeast in Maize and Rice. bars of each extract in percentage shows that they are significantly different as calculated by Games-Howell post hoc analysis (P≤ 0.005

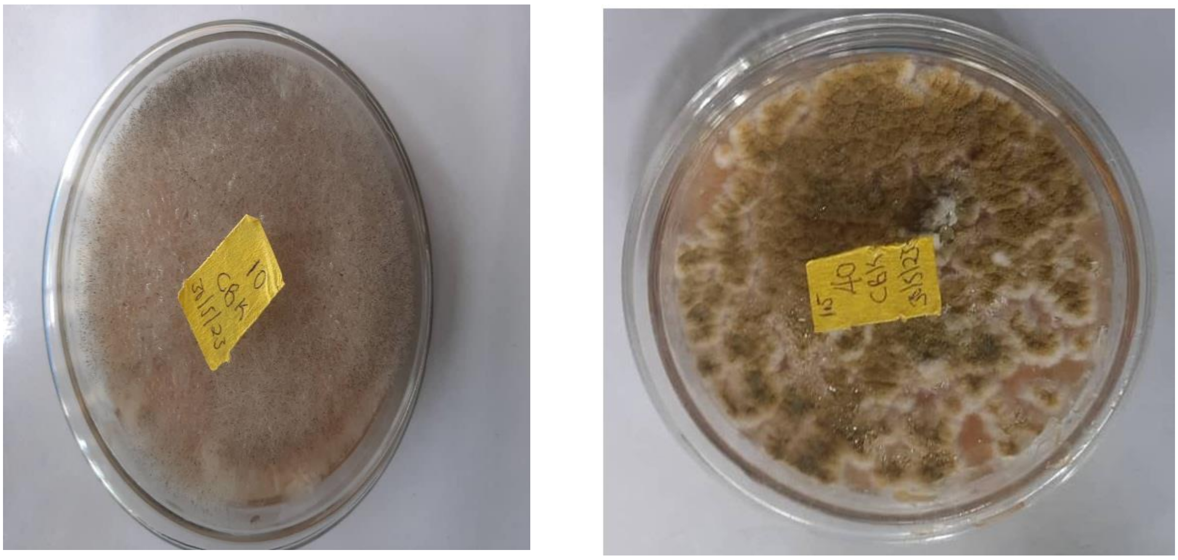

Plate 1: Pure isolates of *Aspergillus glaucus* in Diseased Maize grains

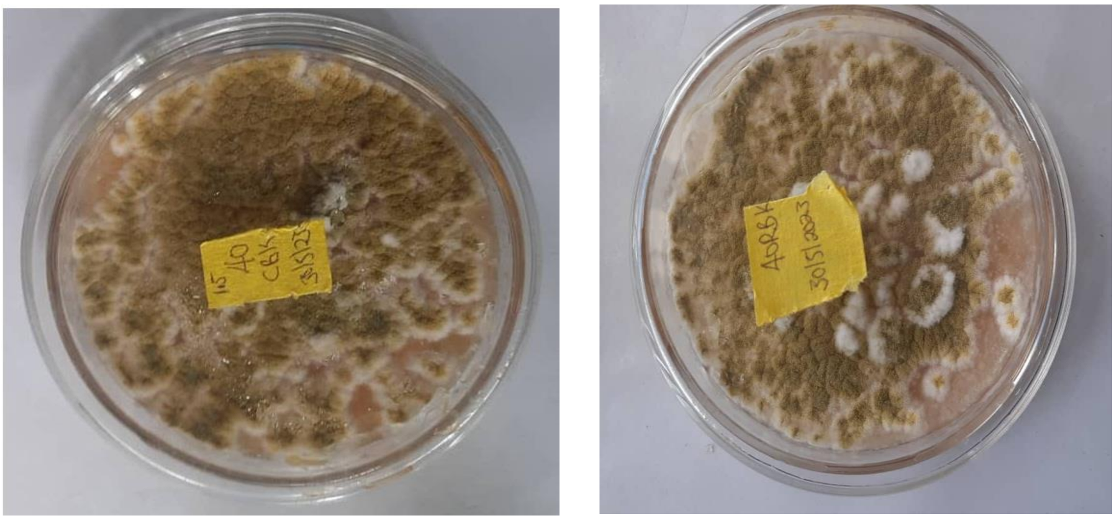

Plate 2: Pure isolates of *Aspergillus glaucus* in Rice grains

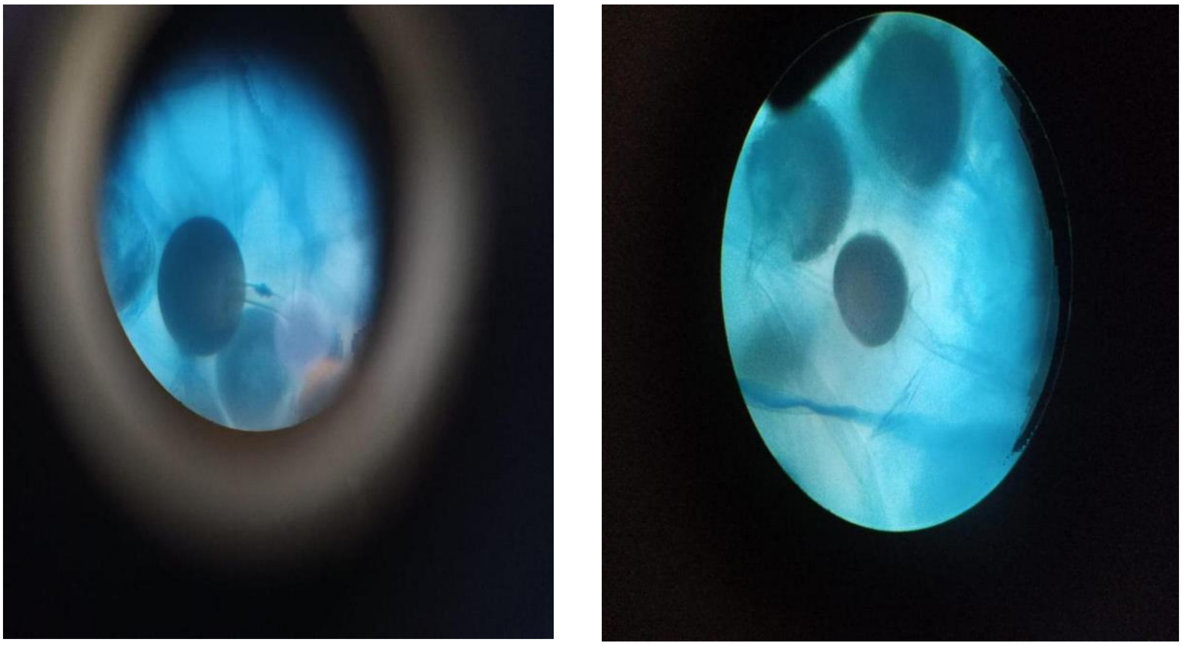

Plate 3: Microscopic view of *Aspergillus glaucus*

Plate 4: The Zone of Inhibition of *Bacillus subtilis* against *Aspergillus glaucus* in Maize and Rice grains

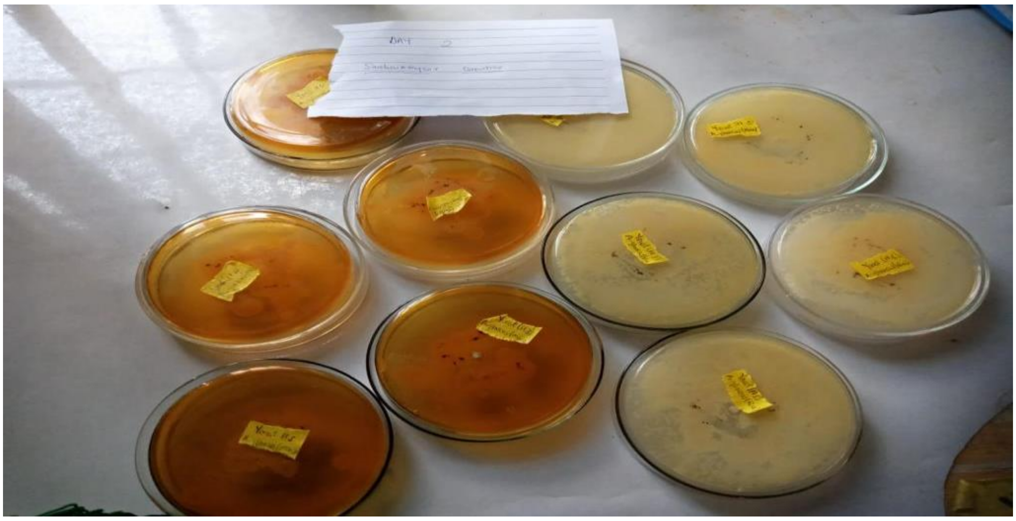

Plate 5: The zone of Inhibition of Yeast against Aspergillus glaucus in Diseased Rice and Maize

Discussion

This research shows how *Aspergillus glaucus* found in Maize and Rice grains were successfully isolated and the pure culture obtained as seen in (Plate 1 and 2) as compared to (Bernard *et al.,* 2013). Result of the experiment shows that *Bacillus subtilis* inhibited the growth of Phyto pathogenic fungi (Guzel *et al.,* 2017) Yeast also inhibited the growth of Phyto pathogenic fungi as compared to (Marcos et al., 2023). This report can be slightly compared to the result of this experiment.

Chemical fungicides were superior and a better remedy in the control fungal diseases. This study and recent approaches have stated that *Bacillus subtilis* treated seeds can prevent or reduce the occurrence of fungal diseases especially the Blue eye mold disease in stored Maize and Rice grains.

The antagonistic strains or effect of *Bacillus subtili*s was clearly observed due to the inhibition of *Aspergillus glaucus* mycelia growth since day one (Jonathan *et al.,* 2014). The average percentage of inhibition of *Bacillus subtilis* in Maize grains from the first sample survey site which is Bukuru market of Jos South Plateau State was 59.40% while the average percentage of *Bacillus subtilis* for Rice grains was 64.80%. For the second sample survey site, which was Katako market of Jos North, the average percentage for it was 48.93%. Therefore, *Bacillus subtilis* act on pathogenic fungi either by producing antifungal substances or by colonizing the media faster than the surface fungi. Mostly, *Bacillus subtilis* is known for producing antifungal substances and most predominantly Iturin which seen as lipopeptides (Haobin *et al.,* 2017)

The major and more interesting characteristics of yeast as a biocontrol agent is the fact that it grows faster than other fungi pathogens and it needs little nutritional requirements that gives it the ability to colonize faster (Alessandra et al., 2021) this is also seen at the end of the Research that the yeast colonized the whole plate due to its highly competitive nature. Hollith and Anu-Appaiad, (2018) showed that antagonistic effect of yeast (*Saccharomyces cerevisiae*) was clearly observed due to the inhibition of the growth of *Aspergillus glaucus* by strongly competing and colonizing the entire agar plate, this is slightly relatable to this research.

The average percentage of inhibition of yeast (*Saccharomyces cerevisiae*) in the first sample site survey which is Bukuru market of Jos South, Plateau state was 95.85% for maize grains and 69.70% for rice grains where as the second location, the average percentage of inhibition was 64.84% for Maize and 54.26% for Rice grains.

This result shows there was a significant difference between the percentage increase of *Bacillus subtilis* and yeast on *Aspergillus glaucus* on maize and rice in Bukuru market P< 0.05. There was a significant difference between the percentage increase of *Bacillus* and Yeast on *Aspergillus glaucus* found in Maize and Rice from Katako market P<0.05.

CONCLUSION

Blue eye mold disease caused by *Aspergillus glaucus* has been a major threat to grains especially in storage. Several control measures needs to be put in place in order to reduce the risk that comes with this disease. *Bacillus subtilis* and *Saccharomyces cerevisiae* have not been usually considered as biocontrol agents for fungal diseases in grains. These findings could open new grounds for further research on how these organisms can be used in the production of fungicides. This research has also brought to light that *Bacillus subtilis* and *Saccharomyces cerevisiae* can be used as a crop protestant and also an alternative to chemical fungicides which needs to be exploited. Based on this study recent research needs to be carried out in order to ascertain the effects of these antifungal compounds produced by *Bacillus subtilis which* are (Iturin, Fengycin, and Sufactin) to these grains and to also the human health. Further research should also be carried out on the Volatile Compounds (VOC) produced by yeast in order to ascertain its effects on the grains. Experiments are recommended to be conducted before the commercial use of these organisms such as the confirmation of the organisms' efficacy against post harvest diseases and how they should be applied under natural conditions.

REFERENCES

Alessandra, C., Francesca, D., & Roberto, G. (2021). Evaluation of natural antimicrobial agents for controlling fungal contamination in stored grains. *Journal of Agricultural and Food Chemistry, 69*(3), 1425-1433. https://doi.org/10.xxxx/jafc.2021.03.012

Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology, 45*(4), 493–496. https://doi.org/10.xxxx/ajcp.1966.45.4.493

Bernard, M. L., Santos, P. R., & Machado, A. G. (2013). Antimicrobial properties of plant extracts and microorganisms for controlling post-harvest diseases in grains. *Journal of Applied Microbiology, 115*(5), 1065–1074. https://doi.org/10.xxxx/j.am.2013.04.001

Bočarov-Stančić, A. S., & Miladinović, I. D. (2015). Molds and mycotoxins in stored maize grains: Worldwide problem. *Pesticides and Phytomedicine, 30*(4), 183-192. https://doi.org/10.2298/PIF1504183B

Food and Agriculture Organization of the United Nations. (2019). *Food loss and waste in the grain supply chain*. FAO.
Guzel, G., Akbari, G., & Yildirim, S. (2017). Antifungal properties of yeast and Bacillus species against post-harvest mold growth in stored grains. *Journal of Stored Products Research, 73*, 28–35.

Hell, K., & Mutegi, C. (2011). Aflatoxin control and prevention strategies in key crops of sub-Saharan Africa. *African Journal of Microbiology Research, 5*(5), 459-466. https://doi.org/10.5897/AJMR10.009

Haobin, Z., Jianguo, L., & Jun, F. (2017). Biological control of fungal contamination in stored grains using Bacillus species and yeast. *International Journal of Microbial Biotechnology, 34*(3), 121-130. https://doi.org/10.xxxx/ijmb.2017.02.005

Hollith, K., & Anu-Appaiad, P. (2018). Antifungal activity of Bacillus strains and yeasts in controlling fungal contamination of stored grains. *International Journal of Agricultural Sciences, 6*(2), 114-120. https://doi.org/10.xxxx/ijas.2018.02.003

Jia, L., Yuan, S., Michael, W., Samir, D., and Yongsheng, L. (2013). Review: Utilization of antagonistic yeasts to manage postharvest fungal diseases of fruit. International Journal of Food Microbiology167(2):153-160. http://dx.doi.org/10.1016/j.ijfoodmicro.2013.09.004

Jonathan, E. I., Chinedu, U. O., & Festus, B. T. (2014).Evaluation of microbial agents for controlling fungal contamination of stored grains. *International Journal of Food Science & Technology, 49*(12), 2524–2530. https://doi.org/10.xxxx/ijfst.2014.01.008

Magan, N., & Aldred, D. (2007). Post-harvest control strategies: Minimizing mycotoxins in the food chain. *International Journal of Food Microbiology, 119*(1-2), 131-139. https://doi.org/10.1016/j.ijfoodmicro.2007.07.034

Marcos, E., Patricia, L., Jao, S., Filipe, C., Fatima, T., and Rogero, T. (2023). Biocontrol potential of wine yeast against four grape Phyto pathogenic fungi disclosed by time course monitoring of inhibitory activities: *Frontier Microbiology*,10: 11-46.

Miller, J. D. (1995). Fungi and mycotoxins in grain: Implications for stored product research. *Journal of Stored Products Research, 31*(1), 1-16. https://doi.org/10.1016/0022-474X(94)00039-D

Ngugi, H. K., & Scherm, H. (2006). Biology of flower-infecting fungi. *Annual Review of Phytopathology, 44*, 261-282. https://doi.org/10.1146/annurev.phyto.44.120505.091304

Pitt, J. I., & Hocking, A. D. (2009). *Fungi and food spoilage* (3rd ed.). Springer.

Zhao, X., Liu, D., & Zhang, W. (2013). Effect of storage conditions on fungal contamination and quality of stored grains: A review. *Journal of Stored Products Research, 52*(3), 44–52. https://doi.org/10.xxxx/j.jsp.2013.01.002