*Biological Forum – An International Journal* **13(3a): 47-52(2021)**

**ISSN No. (Print): 0975-1130**
**ISSN No. (Online): 2249-3239**

# Development of Yeast Based Biocontrol Formulation for Post Harvest Disease Management with Osmotic and Oxidative Stress Tolerance

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*(Received 19 June 2021, Accepted 02 September, 2021)*
*(Published by Research Trend, Website: www.researchtrend.net)*

**ABSTRACT: The yeast based biocontrol product is safe alternative to synthetic chemicals against post-harvest diseases. Antagonistic yeasts are exposed to a wide range of adverse stresses during pre and post-harvest application, as well as during the production process. Tolerance to environmental stresses is a prerequisite for successful development of biocontrol yeast formulation. An investigation was carried out to develop a stable liquid formulation of the antagonistic yeast isolate Sc YZ 7 (*Saccharomyces cerevisiae*) with high shelf life and osmotic and oxidative stress tolerance capacity. Osmotic stress tolerance of Sc YZ 7 yeast isolate was evaluated by changing the concentration of molasses and also with different concentrations of Sodium chloride in molasses urea medium. The number of viable cells (CFU $ml^{-1}$) was determined using modified serial dilution method. The formulation prepared with yeast isolate had highest viability in 1% NaCl and 15% molasses added separately upto 120 days of storage whereas, in control with 5% concentration the viability drastically reduced. The formulations were exposed to oxidative stress by adding $H_2O_2$ at different doses. The formulation containing 5% sorbitol + 1mM ascorbic acid and 5% sorbitol+5% glycerol maintained the viability upto 60 mM $H_2O_2$ concentration. The formulations were exposed to both osmotic and oxidative stress and the presence of additives like sorbitol and ascorbic acid showed a protective effect against stresses. The findings suggest that the development of a cheap yeast-based biocontrol formulation with high osmotic and oxidative stress tolerance capacity and a longer shelf life for post-harvest disease management on a commercial scale is possible in the presence of some protective additives.**

**Keywords:** Antagonistic Yeast, Osmotic stress, Oxidative stress, Biocontrol formulation, ecofriendly management.

## INTRODUCTION

Postharvest decay of fruits by fungal pathogens is mostly managed by synthetic chemical fungicides to reduce significant economic losses. However, consumer health concern and environmental impact of synthetic fungicides prompted to develop safe alternative methods (Droby *et al.,* 2009). Post-harvest application of biocontrol agents has been very successful because of the controlled environment which exists during processing, package and storage which is promising to application and establishment especially on the perishable surfaces. Yeasts are unicellular naturally occurring microbial population which is identified to control many postharvest pathogens (Freimoser *et al.,* 2019). There are reports of antagonistic property of yeasts which includes Anthracnose of mango (Kefialew and Ayalew 2008), Blue mould of pear (Torres *et al.,* 2006), Anthracnose of banana, Decay of Litchi (Zhimo *et al.,* 2016) etc. Yeast based technologies and products (e.g Aspire® containing *Candida oleophila*, Yield Plus® containing *C. albidus*) for postharvest disease management of fruits are already practiced and developed in Europe. However, there are no commercially available formulations in India.

Formulation is an important step in developing a bio control product. The successful delivery of biocontrol agents, shelf life, stability and effectiveness in commercial conditions depend on the formulation. Both pre- and post-harvest application, as well as the production process, exposes antagonistic yeasts to a wide range of adverse stresses that can affect their viability and efficacy. Sui *et al.,* (2015) recently reviewed the published research on the response of yeast biocontrol agents to a variety of environmental stresses including osmotic and oxidative stresses. Tolerance to such environmental stresses is a prerequisite for successful development of biocontrol yeast formulation. When they apply on the host surface, osmotic stress is significant. When yeast cells are subjected to osmotic stress, they experience rapid outflow and death. As a result, when selecting and developing a potential biocontrol strain, the ability to respond to the osmolarity of the external environment should be taken into account. The yeast cells adapted to a changed stress physiologically by increasing the levels of trehalose and, in particular, glycerol (polyols) as significant compatible osmolytes, implying that they play a role in osmotic stress defence (Sharma and Sharma 2017). Production and accumulation of Reactive Oxygen Species (ROS) in wound and during early phase of colonization of the pathogen in the fruit tissue is a common natural process which can adversely

affect the yeast wound competency (Sharma *et al.*, 2009). *C. oleophila* and another biocontrol yeast, *Metschnikowia fructicola*, generated high levels of ROS when applied to intact or wounded apple fruits (Macarisin *et al.*, 2010). Thus, enhancing the oxidative stress tolerance of biocontrol yeasts may be a useful approach to improve the controlling activity. A study conducted by Ming *et al.*, (2020) indicated that both the 0.1 M mannitol and 0.1 M sorbitol treatments improved the tolerance of *Debaryomyces hansenii* to subsequent oxidative and high-temperature stress.

Several studies have shown that the survival of yeast cells was significantly higher in molasses urea based formulation (Mukhtar *et al.*, 2010; Sokchea *et al.*, 2018). Moreover, it is economically feasible for industrial production. Based on the above background the current study was aimed todevelop a cheap Yeast based biocontrol formulation with high osmotic and oxidative stress tolerance capacity and longer shelf life for post-harvest disease management at commercial scale.

## MATERIALS AND METHODS

**Biocontrol yeast and Test pathogen:** The yeast isolate *Saccharomyces cerevisiae* (ScYZ7; NCBI accession no- KT459474) was obtained from Departmental Culture collection of Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal with known biocontrol activity against *Colletotrichum musae* causing anthracnose of banana was used for studying the antagonistic potential of the yeast-based formulation (Zhimo *et al.*, 2016).

**Media selected for Mass Multiplication of Yeast Cells:** Molasses Urea media (Cane Molasses, 50 g/l; Urea, 1.2 g/l) were used for multiplication and formulating the yeast cells. CFU/ml was counted using haemocytometer.

**Evaluation of Osmotic Stress Tolerance of the Yeast Formulations:** The tolerance of osmotic stress of yeast *Saccharomyces cerevisiae* YZ 7 was evaluated in Molasses urea media (cane molasses, $50gL^{-1}$; urea, $1.2gL^{-1}$) at pH 6.5 at storage temperature of 28 ± 1°C. The following concentrations of cane molasses in the molasses urea media were used to evaluate the sugar stress tolerance: 5%, 15%, 20%, 25%, 30%, 35%, 40%, and 45%. The salt tolerance was evaluated by adding different concentrations of Sodium chloride at the rate of 1%, 1.5%, 2%, and 2.5% in the formulation.

In molasses urea media, fresh yeast cells were grown for 72 hrs as starter culture in 250ml Erlenmeyer flask and after the yeast cells reached exponential phase about 1 ml aliquots of this suspension were added to each flask containing different concentrations of molasses and salt aseptically. The concentration of yeast cells in aliquots was at $1.4 \times 10^8$ CFU $ml^{-1}$ and added in replicated flasks stored at 28 ± 1°C. The number of viable cells (CFU $ml^{-1}$) was determined using modified serial dilution method by taking 10µl (composite sample of replicated flasks) aliquots from each formulation and plating on YPDA (PDA media supplemented with (1%) yeast extract (1g/l) plates after 4, 10, 15, 20, 30, 60, and 120 DAS (Thomas *et al.*, 2015).

**Evaluation of Oxidative Stress Tolerance of Yeast ScYZ-7 Formulations:** Formulations of Sc YZ7 in MU media were prepared (cane molasses, 50g $L^{-1}$; urea, 1.2g $L^{-1}$) at pH 6.5 at storage temperature of 28 ± 1°C. The cell count was calculated by haemocytometer and adjusted to a minimum of $1.2 \times 10^8$ CFU $ml^{-1}$. The suspension was distributed equally to ten 50ml conical flasks. A 100mM stock solution of $H_2O_2$ (50%) was prepared and working standards of concentrations 0mM, 20mM, 40mM and 60mM were made using the equation $N_1V_1=N_2V_2$. Control set received no stress. The oxidative stress tolerance of the yeast isolate grown in molasses urea media with additives, Sorbitol (5%) + glycerol (5%), and sorbitol (5%) + Ascorbic acid (1mM) were evaluated. The oxidative stress tolerance of the yeast isolate grown in molasses urea media without any additives was also evaluated. The flasks were kept in an incubator shaker at 28±1°C at 150rpm for 24 hours and after that stored for 30 days at 28±1°C. The treated suspensions were serially diluted up to $10^{-6}$ and plated on YPDA to count the colonies after exposure to oxidative stress at 4, 15 and 30 DAS by modified serial dilution method.

**Evaluation of Osmotic and Oxidative Stress Tolerance of Yeast ScYZ-7 Formulations**

The yeast formulation which has shown highest viability against osmotic stress (15% molasses) was selected and its efficiency against different concentrations of $H_2O_2$ were evaluated. Control set received no stress. In molasses urea media, A starter culture with yeast population at 1-1.4 $\times 10^8$ CFU $ml^{-1}$ was prepared and fresh yeast cells were grown for 72 hr as starter culture in 250ml Erlenmeyer flask and after the yeast cells reached stationary phase about 1 ml aliquots of this suspension were added aseptically to each flask containing 15% cane molasses urea formulation and stored at 28 ± 1°C. After 3 days of storage, the yeast cells were at the starting of their exponential phase at which 5% sorbitol+5% Glycerol, 5% sorbitol+1 mM Ascorbic acid were added in different flasks containing 15% molasses urea formulation. After 3 days $H_2O_2$ was added as oxidative stress inducer at different concentrations 0Mm, 20Mm, 40mM, and 60 mM. The preparation of stock solution and working standards has been mentioned in the previous section. The treated suspensions were serially diluted up to $10^{-6}$ and plated on YPDA to count the colonies after exposure to oxidative stress at 0, 1 and 2, 3 and 10 DAS by modified serial dilution method.

**Modified Serial Dilution Method:** Media was prepared and poured in the pre sterilized petriplates (15-20 ml per 9 cm diameter). Serial dilutions in sterilized distilled water upto $10^{-6}$ were prepared. The micro-tips were changed after each dilution. Reverse side of the petriplates were marked.10µl aliquots from the dilutions were applied as micro-drops in marked areas. Plating was started from the last dilution and using the same tip. The plates were allowed to dry in the LAF cabinet for 4-6 minutes. Plates were sealed and incubated at 28±1°C for 24 hrs (Fig. 1). Colonies were

counted in each sector and the CFU $ml^{-1}$ of the sample was worked out by applying the formula: $n \times 10^{d+2}$ where, n = number of colonies in 10µl sample; d = dilution level yielding countable colonies.

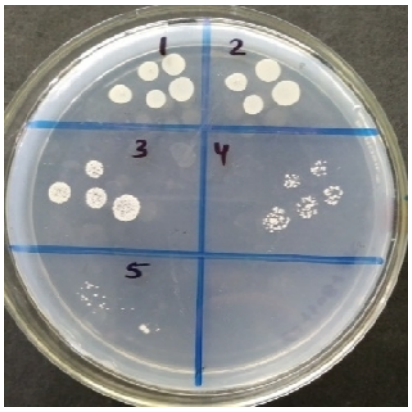


**Fig. 1.** Dilution plating for counting CFU $ml^{-1}$ using modified serial dilution plating method.

**Data analysis:** The lab experiments were arranged in Completely Randomized Design (CRD). Average of the measurement of the cells was taken and Standard deviation (SD) and Coefficient of variance (CV) were calculated using Microsoft Excel. Replicated data on viability of yeast cells in osmotic stress, oxidative stress tolerance, osmotic and oxidative stress tolerance with additives were subjected to analysis of variance (ANOVA) using OPSTAT and Microsoft Excel software.

## RESULTS AND DISCUSSION

### Evaluation of osmotic stress with salt on yeast in the formulation

The cell number of Sc YZ-7 in molasses urea media containing salt (NaCl) at different concentration *viz* 1%, 1.5%, 2%, 2.5% was improved by varying degrees in all the concentrations compared to the population in molasses urea formulation without NaCl which kept as control at 28±1 °C (Table 1). The cell number/ml increased upto 20 days of storage at 28°C and maintained the cell count upto 120 days of storage. The highest cell number was obtained with initial count of $3.2 \times 10^6$ CFU $ml^{-1}$ in the formulation containing 1% NaCl ($5.10 \times 10^7$ CFU $ml^{-1}$) as additive and it was on par upto 2% salt concentration whereas the cell number in the control formulation drastically reduced to $1.31 \times 10^3$ CFU $ml^{-1}$ after 4 months of storage at 28°C. It indicates that Sc YZ 7 shows highest number of cells upto 2% salt even after 120 days of storage. In control formulation, the number of cells increased upto 20 days of storage and then decreased. This may be due to the depletion of nutrients in the formulation. Even though the formulations with salt observed with low growth rate, they showed high cell population after 4 months of storage. It indicates that under stress conditions, the cells preserve nutrients and accumulate some compatible solutes as osmoprotectants. However, it was observed that during the experiment, the cell number decreased with an increased concentration of salt.

**Table 1: Population of *Saccharomyces cerevisiae* YZ 7 in Molasses Urea medium based liquid formulation with different concentration of NaCl as osmotic stress agent at different days after storage (DAS) at 28 ±1 °C.**

| Salt (NaCl) concentration (%) | Population of yeast cells ($Log_{10}$ CFU $ml^{-1}$) at days after inoculation | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 4 | 10 | 20 | 25 | 35 | 60 | 120 |
| 1.0 | 6.50* | 7.70 | 7.80 | 7.75 | 7.77 | 7.70 | 7.69 | 7.71 |
| 1.5 | 6.50 | 7.66 | 7.69 | 7.61 | 7.59 | 7.57 | 7.56 | 7.64 |
| 2.0 | 6.50 | 7.53 | 7.44 | 7.57 | 7.52 | 7.55 | 7.57 | 7.62 |
| 2.5 | 6.50 | 7.11 | 7.20 | 7.51 | 7.41 | 7.44 | 7.50 | 7.58 |
| Control | 6.50 | 8.07 | 8.09 | 7.82 | 7.75 | 7.75 | 7.72 | 3.12 |
| SEM± | | 0.015 | 0.023 | 0.008 | 0.023 | 0.020 | 0.012 | 0.033 |
| C.D (5%) | | 0.044 | 0.070 | 0.023 | 0.081 | 0.061 | 0.036 | 0.100 |

*Values are means of four replications

**Evaluation of osmotic stress with Molasses on yeast in the formulation:** The cell number of Sc YZ-7 in the formulation containing molasses upto 45% were observed till 120 days of storage at 28±1 °C (Table 2). There were significant differences in the number of yeast cells in all treatments with additional molasses (more than 5% molasses) as osmotic stress agent compared to control (5% molasses). As the quantity of molasses increases upto 20% in the formulation, the number of yeast cells has been increased and following high molasses concentrations showed a drastic reduction in the number of cells after 4 days of storage. It has been observed that the number of cells in the formulation containing molasses more than 40 % reduced to zero after 10 days of storage. There was drastic reduction in the population of yeast cells to zero in 30% sugar formulation after 15 days of storage. The experiment also showed that yeast cells can tolerate osmotic stress which is induced by increase in molasses concentration in the formulation upto 25% even after 4 months of storage. In 25% sugar concentration, the number of cells decreased to $3.6 \times 10^6$ after 20 days of storage. But it has been increased after 25 days of storage and the maintained the count ($3.82 \times 10^7$) even at 120 days of storage at 28°C. This might be because of the multiplication of yeast cells which has been adapted to higher osmotic stress. In the present study, it was found that the yeast cells formulated with 15% sugar concentration showed more cell number ($4.24 \times 10^7$ CFU $ml^{-1}$) compared to control ($1 \times 10^3$ CFU $ml^{-1}$) after 4 months of storage 28°C.

**Table 2: Population of *Saccharomyces cerevisiae* YZ 7 in Molasses Urea medium based liquid formulation with different concentration of molasses as osmotic stress agent at different days after storage (DAS) at 28 ±1 °C.**

| Sugar concentration (%) | Population of yeast cells ($Log_{10}$ CFU $ml^{-1}$) at days after inoculation | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 4 | 10 | 15 | 20 | 25 | 60 | 120 |
| 15 | 6.50 | 8.06 | 7.89 | 7.92 | 7.85 | 7.68 | 7.67 | 7.63 |
| 20 | 6.50 | 8.12 | 7.81 | 7.71 | 7.80 | 7.66 | 7.65 | 7.60 |
| 25 | 6.50 | 8.05 | 7.75 | 7.60 | 6.56 | 6.70 | 7.54 | 7.58 |
| 30 | 6.50 | 7.75 | 6.64 | 0 | 0 | 0 | 0 | 0 |
| 35 | 6.50 | 6.75 | 5.30 | 0 | 0 | 0 | 0 | 0 |
| 40 | 6.50 | 6.12 | 0 | 0 | 0 | 0 | 0 | 0 |
| 45 | 6.50 | 6.10 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 (Control) | 6.50 | 8.07 | 8.09 | 7.82 | 7.78 | 7.75 | 7.72 | 3.12 |
| SEM± | | 0.011 | 0.001 | 0.006 | 0.012 | 0.012 | 0.008 | 0.038 |
| C.D (5%) | | 0.034 | 0.032 | 0.020 | 0.037 | 0.035 | 0.025 | 0.115 |

*Values are means of three replications

The evaluation of osmotic stress tolerance on yeast cells in the formulation shows a comparative result with Logothetis *et al.,* (2007) who evaluated the growth and viability of *S. cerevisiae* (VIN 13) in the presence of 0%, 4%, 6%, 10% w/v NaCl in glucose-based defined medium. The viability remained longer at higher concentrations of salt. But cell multiplication rate decreased proportionally with increased salt concentration. Higher osmotic stress conditions (especially 10% NaCl) maintained a high viability over time compared with that of control. Abdel and El-Moghaz (2010) also observed the similar result where the growth of cells was reduced with increased of concentration of stress agent from (0.5M to 1.5M) and found that *Pichia pastoris* is more tolerant to high concentration of NaCl and sorbitol than *Saccharomyces cerevisiae*). Compatible solutes help in survival under extreme osmotic conditions either by maintaining osmotic balance by increasing their concentration to avoid dehydration or by maintaining membrane fluidity and stabilizing cellular proteins by keeping them hydrated (Wani *et al.,* 2013). A study on physiological basis for the tolerance of yeast *Zygosaccharomyces bisporus* to salt stress was accompanied by an upsurge in the level of cellular metabolites such as trehalose (reserve carbohydrate) and chiefly glycerol (polyols) as major compatible osmolytes, suggesting their role in defense mechanism against osmotic stress (Sharma and Sharma 2017).

**Evaluation of oxidative stress tolerance of yeast YZ-7 with different additives:** The experiment was conducted by growing the yeast cells in different formulations. At the starting of their exponential phase, they were exposed to different concentrations of $H_2O_2$ (0, 20, 40 and 60 mM) and stored for 30 days after exposure. The results obtained are presented in Table 3. It was observed that yeast population gradually decreased with increasing $H_2O_2$ concentration of storage till 30 days of storage. The formulation containing 5% sorbitol + 1mM Ascorbic acid and 5% sorbitol + 5% glycerol maintained more than equal to 95% of the initial cell number even at 60 mM after 4 days of storage. There were significant differences in the cell number in all the treatments with different concentration of $H_2O_2$ stress compared to control.

At 20 mM, the cell number was highest in the media with sorbitol and Ascorbic acid at 4 DAS ($8.82 \times 10^6$ CFU $ml^{-1}$) which was *on par* with the media with sorbitol and glycerol, and 30 DAS ($4.52 \times 10^6$ CFU $ml^{-1}$). At 40 mM concentration, the cell number was highest in the media with sorbitol and Ascorbic acid at 4 DAS ($9.45 \times 10^6$ CFU $ml^{-1}$), 18 DAS ($7.15 \times 10^6$ CFU $ml^{-1}$), and 30 DAS ($3.2 \times 10^6$ CFU $ml^{-1}$). At 60 mM concentration also the formulation supplemented with Sorbitol and Ascorbic acid maintained higher cell count. From the experiment it has been observed that there is no significant difference in the cell number between ascorbic acid and glycerol in combination with sorbitol at different concentrations of $H_2O_2$. However, the presence additives like sorbitol, glycerol and Ascorbic acid have some protective effect on cells against $H_2O_2$ stress.The additives used increased the stress tolerance capacity of yeasts enabling the cells to multiply even when exposed to higher stress.

**Table 3: Population of *Saccharomyces cerevisiae* YZ 7 in different formulation following hydrogen peroxide (mM) exposure at different days after storage (DAS) at 28 ±1 °C.**

| Treatments | Population of yeast cells ($Log_{10}$ CFU $ml^{-1}$) days after treatment | | |
|---|---|---|---|
| | 4 | 18 | 30 |
| 0mM (control) | 8.12* | 7.84 | 7.81 |
| 20mM | 5.46 | 6.48 | 6.50 |
| 40mM | 4.83 | 6.57 | 6.51 |
| 60mM | 4.57 | 0.00 | 0.00 |
| 20mM + Sorbitol + Glycerol | 6.92 | 7.64 | 7.50 |
| 20mM + Sorbitol + Ascorbic acid | 6.95 | 7.62 | 7.66 |
| 40mM + Sorbitol + Glycerol | 6.87 | 7.57 | 7.73 |
| 40mM + Sorbitol + Ascorbic acid | 6.98 | 7.85 | 7.93 |
| 60mM + Sorbitol+Glycerol | 6.86 | 7.84 | 7.72 |
| 60mM + Sorbitol + Ascorbic acid | 6.62 | 7.74 | 7.91 |
| SEM± | 0.032 | 0.017 | 0.098 |
| C.D (5%) | 0.097 | 0.050 | 0.291 |

*****Values are means of three replications.

Initial concentration at day 0 = 7.2 ($Log_{10}$ CFU $ml^{-1}$) in the formulations without any additives and 7.5 ($Log_{10}$ CFU $ml^{-1}$) with additives which means $H_2O_2$ at different concentrations added in the formulation and cells were at the starting of their exponential phase.

Liu *et al*., (2011) investigated the survival of *Cryptococcus laurentii* and *Pichia membranifaciens* in liquid formulations with sugar protectants (trehalose and galactose) and L-ascorbic acid during storage at 4°C and 25°C. When galactose or trehalose was used alone as protectant, *C. laurentii* maintained relatively high viability in potassium phosphate buffer. Another study of Li *et al.,* (2014) also supports the protective effect of Ascorbic acid in which it was found that ascorbic acid treated cells of *P. caribbica* showed increase in viability and the intracellular ROS accumulation was also significantly lower as compared to untreated cells. Ming *et al.,* (2020) reported the effect of exogenous mannitol and sorbitol on the viability of the antagonist yeast, *Debaryomyces hansenii* by expose them to oxidative and high-temperature stress and found that both mannitol and sorbitol treatments improved the tolerance of *Debaryomyces hansenii* to subsequent oxidative and high-temperature stress.

**Evaluation of Osmotic and Oxidative Stress Tolerance of Sc YZ7:** The formulation which maintained highest cell number under sugar stress (with 15% molasses) was selected and tested against different concentration of $H_2O_2$. The number of yeast cells on 0, 1, 2, 3 and 10 days after storage (DAS) are presented in Table 4. It was observed that the number of yeast cells/ml was decreasing with increasing $H_2O_2$ concentration upto 10 days of storage. It was found that the effect of stress has been prolonged upto 2 days after the exposure and then the adapted cells start to multiplying with slow growth rate. After 1 day of storage, the formulation containing sorbitol and Ascorbic acid maintained 98.9% of their initial population at 20 mM. Under no stress condition, the cell number was higher in the formulations with sorbitol, sorbitol + Ascorbic acid upto 10 days of storage. But the formulation with sorbitol + Ascorbic acid retained at the initial value even after 10 days of storage. This indicates that Ascorbic acid has some growth promoting effect on the cells. At 60 mM concentration, the cell number was highest in the formulation with sorbitol at 1DAS ($1.1 \times 10^3$ CFU $ml^{-1}$), 2DAS ($1.25 \times 10^3$ CFU $ml^{-1}$), 3DAS ($4.7 \times 10^4$ CFU $ml^{-1}$) and 10 DAS ($2.84.7 \times 10^6$ CFU $ml^{-1}$) compared to the formulation with Sorbitol + Ascorbic acid. After 10 days of storage, the cell number was highest in formulation supplemented with sorbitol and the population increased to $3.98 \times 10^7$ CFU $ml^{-1}$ as compared to the population before exposure to stress $3.15 \times 10^7$ CFU $ml^{-1}$ at 40 mM concentration. In general, this study found that at lower concentration of $H_2O_2$ stress Ascorbic acid shows some protective role along with sorbitol on the cells but at higher concentrations ascorbic acid does not shows additional protective effect.

The supplementation of the compatible solute sorbitol not only promoted cell growth but also increased the ethanol fermentation capability of *Zymomonas mobilis* under heat, ethanol, and osmotic stresses (Sootsuwan *et al.*, 2013). The responses to oxidative and osmotic stress differ significantly, yet they also overlap. Osmotic stress causes overproduction of reactive oxygen species (ROS), many antioxidant functions are highly inducible upon osmotic stress, and external addition of antioxidants rescues the sensitivity of yeast cells to osmotic stress (Eardley and Timson 2020). Rienzo *et al.,* (2012) has been reported an induction of the osmostress-sensing HOG pathway by some oxidative treatments. Induced sugar metabolism is also observed when plants are subjected to short periods of oxidative or osmotic stress, suggesting that soluble sugars may function as osmoprotectants during stress (Zanella *et al.,* 2016).

**Table 4: Population of *Saccharomyces cerevisiae* YZ 7 in different formulation with osmotic stress following hydrogen peroxide exposure at different days after storage (DAS) at 28±1°C.**

| Treatments | Population of yeast cells ($Log_{10}$ CFU $ml^{-1}$) days after treatment | | | | |
|---|---|---|---|---|---|
| | 0** | 1 | 2 | 3 | 10 |
| Sorbitol + 20mM $H_2O_2$ | 7.51 | 6.41* | 6.48 | 6.44 | 6.82 |
| Sorbitol + Ascorbic acid 1Mm + 20mM $H_2O_2$ | 7.55 | 7.47 | 6.19 | 6.48 | 7.39 |
| Sorbitol + 40mM $H_2O_2$ | 7.51 | 5.42 | 5.68 | 6.30 | 7.60 |
| Sorbitol + Ascorbic acid 1mM + 40mM $H_2O_2$ | 7.55 | 4.71 | 6.47 | 6.23 | 7.36 |
| Sorbitol + 60mM $H_2O_2$ | 7.51 | 4.05 | 4.10 | 4.67 | 6.45 |
| Sorbitol + Ascorbic acid 1mM + 60mM $H_2O_2$ | 7.55 | 3.80 | 3.73 | 3.78 | 6.20 |
| Sorbitol + Control | 7.51 | 7.54 | 7.57 | 7.56 | 7.32 |
| Sorbitol + Ascorbic acid 1mM + Control | 7.55 | 7.52 | 7.47 | 7.47 | 7.47 |
| SEM± | | 0.028 | 0.039 | 0.022 | 0.031 |
| C.D (5%) | | 0.086 | 0.117 | 0.066 | 0.093 |

*Values are means of three replications. 0**means the day at which cells were exposed to $H_2O_2$ stress at different concentrations in the formulation containing additives and cells were at the starting of their exponential phase

## CONCLUSION

The yeast isolate Sc YZ7 (*Saccharomyces cerevisiae)* was found to be resilient to both osmotic and oxidative stress in the formulation in this study. As a result, it could be employed as a biocontrol stain in the creation of formulations. The adaptation to the stressed environment may be due to the accumulation of antistress compounds in the cells. The presence of additives like sorbitol, glycerol and ascorbic acid showed some protective effect against stresses. The molasses urea based medium is good for yeast cell mass multiplication and is inexpensive enough to be employed in commercial production. It is an

environmentally friendly method of managing post-harvest fungal diseases. A detailed study on physiological changes and metabolism of yeast cells during osmotic and oxidative stresses will contribute the development of yeast based biocontrol formulation with longer shelf life as well as provide insight into antistress compounds.

**Acknowledgement.** We are grateful to the faculty of Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur for their support.

**Conflict of Interest.** There are no conflict of interests to declare to publish this article.

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**How to cite this article:** Thomas, J., Halsana, S., Baral, D. and Saha, J. (2021). Development of Yeast Based Biocontrol Formulation for Post Harvest Disease Management with Osmotic and Oxidative Stress Tolerance. *Biological Forum – An International Journal*, *13*(3a): 47-52.