Original Research Article

Extracted trans-resveratrol from Arachis *hypogaea* enhances expression of sirtuin gene and replicative life span in Saccharomyces *cerevisiae*

ABSTRACT

Aim: Biotic stress given by Aspergillus *niger* enhances trans-resveratrol production in Arachis hypogaea plant. This plant extract increases sir2 gene expression and Replicative Life Span in *Saccharomyces cerevisiae.*

Design of study: Peanut plant was grown in aseptic environment, infected by Aspergillus niger. Plant extract used for quantification of trans-resveratrol by RP-HPLC. Yeast culture was grown in Potato dextrose media along with plant extract. Sir2 gene expression fold calculated by real time pcr. Replicative Life Span of yeast was measured by spectrophotometer.

Place and duration of study: Allele Life Sciences Pvt. Ltd., Department of Biotechnology between February 2017 to March 2020.

Methodology: Biotic stress in *Arachis hypogaea* plant was induced by wounding the leaves and introducing *Aspergillus niger* to enhance trans-resveratrol production. Tran-resveratrol was quantified by Reverse Phase High Pressure Liquid Chromatography (RP-HPLC). Two methods conducted to check reverse aging, the first one ~~epigenetic~~ based on epigenetic, where~~n~~ extracted trans-resveratrol, from infected *Arachis hypogaea* plant extract, added to *S. ~~accharomyces~~ cerevisiae* culture~~, it~~ enhanced expression of Sir2 gene in *S~~accharomyces,~~ cerevisiae* measured by qPCR, ABI applied biosystem. Process included RNA isolation and cDNA synthesis and thereafter qPCR. Enhanced expression of sirtuin responsible for gene silencing as sirtuin (Sir2 gene product) is a class of Histone deacetylase transferase enzyme. The ~~S~~second method, Replicative Life Span of *S. ~~accharomyces~~ cerevisiae* culture increased when Aspergillus niger infected peanut plant extract was added to yeast culture. It ~~which~~ was measured through spectrophotometer at 600 nm and showed high absorbance value.

Result: Trans-resveratrol was quantified by Reverse Phase High Pressure Liquid Chromatography (RP-HPLC) and yield was 2.24 mg/g. Sir2 gene expression increased by 1.56 fold in yeast grown in infected peanut plant extract. Absorbance of yeast culture grown in infected peanut plant extract was 0.522±0.008 which was higher than control.

Conclusion: Sir2 gene expression enhances along with replicative life span in yeast in presence of peanut plant extract.

Keywords : Replicative Life Span; Sirtuin; Reverse Aging; Peanut; Yeast

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INTRODUCTION

As we age older, our body deteriotes and thus it looses the ability to work. Aged body also adds extra pressure to a family, society and to country. If an aged body works normally to earn livelihood and takes care itself, then no burden on family to take care. A time comes when an age group becomes worthless and looks quite heavy burden not to family but for a country also.

At the same time, no one can ignore idea to remain healthy with all organs intact functioning properly and feel enthusiatic till last breathe in this world. Although deterioation of any living body is the process of life but understanding how living body deterioates and how the body can be brought back in state where body deterioation is possibly less is quite worthy. At juvenile age, we feel very energetic and our body has well worth for doing ~~ethlete~~athlete work but as we grow old, we loss body's such capacity. Understanding what changes have taken up in our body is the first challange to confirm and finding accurate pathways of those changes. Visiting history, we find that our ancestors followed some healthy practices in life style when there were no scientific evidences, will also be verified by this reverse aging theory whether it comes food, exercise or religious customs to keep body calorie restricted (fasting). But lone these practices are having their own grey area such as regular food intake will also results into many free radicals accumulation in body which further damages cell[1], if some opts for regular extreme physical exercises then this also produces free radicals[2] and body will remain in demand of more energy whole day and if we may keep body nutrients restricted by fasting then this state may cause lack of key elements in body which can reduce body's immunity[3]. Above stated problems are not today's problem, but it has become part of curiosity for human being since we understood life.

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When multifaceted decline of cellular and ~~organismal~~organism function occurs over time, is described as aging, and actually causes major risk factor for disease suceptibility[4]. Therefore, present researches are targeting to deaccelerate aging process, and this is how lifespan or healthspan can be prolonged by therapeutics of such diseases.

Molecular biology and epigenetics together give solution of above stated problems by reversing age. Gene expression is controlled by environmental factors which brings body in such condition where gene expression can be ~~made~~ silenced or ~~can~~ enhanced ~~expression~~ to produce desirable amino acids[5]. Naturally, peanut plant contains many polyphenol compounds, resveratrol is one of them. Chemically resveratrol is found in two forms, cis-resveratrol and trans-resveratrol. Trans-resveratrol has gained enough popularity as antiaging compound[6] in recent times, but its bioavailability is very low. Therefore, when peanut plant is infected with fungus (here Aspergillus niger), it is found that trans-resveratrol production increases in peanut plant.

Importance of tran-resveratrol

In many religious traditions, fasting has been a~~in~~ regular practice. Scientific data says fasting is one of the reasons of reverse-ageing which rejuvenates our cells to grow young. Religious Hindu books and Roza in muslims give same sense. If body is made somehow calorie restricted, cells do not reach to senescences ~~or apoptosis~~or apoptosis. But practically, ~~,~~it is not possible to be calorie restricted as body demands energy molecules to do our daily routine work, therefore, we need to see other way to rejuvenate the cells. Groundnuts (*Arachis hypogaea* L.) has two very important anti-aging nutrient compounds one is niacin and the other ~~one~~ is trans-resveratrol[7-8]. Conceptually, if genes which switched on due to stress gained in life of organism, are made to switched off then, non stressed cell gain capacity to increase number of cell division. Biologically, Trans-resveratrol activates SIRTUIN enzyme which is NAD+ dependent. Sirtuin is an Histone Deacetylase~~s~~ enzyme which removes acetyl group from lysine present at Histone to facilitate DNA binding thus causing gene silencing[9]. Thus, the amount of NAD+ is a marker of aging in ~~different~~ animals.

Resveratrol is a Sirtuin Activator

Resveratrol has been actively investigated to enhance sirtuin activity with consequent beneficial effects on aging[10]. Sirtuin delays cellular senescence and extends the organismal lifespan through the regulation of diverse cellular processes. Sirtuin suppresses cellular senescence and it is done mainly mediated through delaying the age-related telomere attrition, sustaining genome integrity and promotion of DNA damage repair. Sirtuin, additionally modulates lifespan by interacting with several lifespan regulating signaling pathways including insulin/IGF-1 signaling pathway, forkhead box O and AMP-activated protein kinase. Since Sirtuin mediates the prolongevity effect of calorie restriction. Many activators of Sirtuin have attracted the attention of researchers to develop therapeutics for age-related diseases[11]. Sirtuins are proteins found in all domains of life. In Baker Yeast, –the first known sirtuin, Sir2 (silent information regulator 2), from which the family derives its name, regulates ribosomal DNA recombination, gene silencing, DNA repair, chromosomal stability and longevity. Sir2 homologues have been studied to see its role in modulating lifespan in worms and flies, and may underlie the beneficial effects of caloric restriction, the only regimen whose effect we have seen in fasting that slows aging and extends lifespan in organism. Considerable attention has gained by sirtuins for their impact on mammalian physiology, since they may provide novel targets for treating diseases associated with aging and perhaps extend human lifespan[12].

Biological platforms are requied for the reseach in anti-aging interventions that may further lead to drug discovery. Improved survival during aging can be well studied at cellular platforms which areis highly compatible and unbiased phenotypicceal screeens. Such screening platform should be economical, simple and reliable and one organism that fulfills such requirement is Saccharomyces cerevisiae, which is commonly known as Yeast, a model for human aging and age related diseases. Many pathways in yeast and humans, that are relevaent for aging and disease, are well conserved[13]. Based on sequence similarity, 30% of the yeast genome is conserved to humans. Yeast carries approximately 6000 genes, out of which 90% have already been charaterized[14].

Test of reverse aging

First Approach by Up regulation of Sir2 gene

Here iIn the present study, budding yeast has been selected as model orgnaismorganism. In this organism we found that SIR2 was regulated up when it isthey are grown in presence of peanut extract which contains tran-resveratrol (Quantified by HPLC). Sir2 up regulation causes sirtuin enzyme availability in cell. As Sirtuin, a class of Histone Deacetyl transferase enzyme, removes N-acetyl from histone causing (+) positive charge on lysine exposed to (-) negative charge of phosphate group of DNA, therefore, part of DNA get tightly packed and then no transcription region is found for expression. In this way, those genes which switch on due to stress over the years, (which actually increased the burden of cell and reduced ability to divide), will switch off ultimately.

Second Approach by Replicative Life Span

Over several hours, ability to divide in fresh liquid media of an aging culture declines with age which can be monitored by measuring the optical density of culture aliquot that has been diluted in fresh media. When, in media peanut extract added, number of yeast cells increase.

MATERIAL AND METHODS

HPLC-grade methanol and ethanol were purchased from Merck (India). Trans-resveratrol (98% purity) was purchased from Cayman Chemical (India). Potato dextrose agar and, Hoagland Solution were prepared at Allele Life Sciences Pvt. Ltd., Noida, UP.

A. Fungal Culture

Aspergillus niger was isolated from waste water previously using potato dextrose agar. It was identified and characterised using 18s rDNA sequence.

***Arachis hypogaea L.* Growth**

Peanut seeds (Om Agro Industry, Bikaner, India) were sown in 3.8 liter pots (16 cm diameter) containing autoclaved mix soil (Add Value Biotech, Gurgaon, India) supplemented with Hoagland Solution. Seeds

germinated in 10 days were grown in an insect-free greenhouse with natural light. The greenhouse temperature was kept at 25–30 ˚C. After one month, seedlings were distributed to one individual per pot. Two months old plants with fully developed leaves were used in all experiments. After sowing the seeds, emergence of seedlings occurred in 20 days . The plant that grew normally, after two months, was taken for further treatment.

B. Aspergillus niger infection

The plants were divided into two groups; control and infected, each containing four plants. The leaves were wounded aseptically using a scissor and *Aspergillus niger* culture was spotted on the wounded area. Each plant was then individually covered with a 4 liter plastic storage bag to provide adequate humidity and temperature conditions for fungal growth and colonization of the plant's leaves. The plants were incubated for three days before the removal of bags. Subsequently, plants were grown with regular exposure of sunlight and water sprinkled whenever soil found dry till 3.5 months.

C. Extraction of Resveratrol from whole plant

At the end of experiment, whole plant was dried in heat incubator for 5 days at 50 ˚C and crushed to fine powder in mortar and pestle. Each powdered sample was weighed (0.5 g) and dissolved in ethanol (50 ml). The mixture was stirred at 250 rpm at 50 ˚C for 5 days. Thereafter it was filtered to remove undissolved particles of larger size followed by centrifugation at 7000 rpm for 10 minutes. The supernatant was collected and used for further analysis. All the extraction steps were performed in dark to prevent photochemical degradation of the constituents.

D. HPLC Analysis of Whole Plant Extract

Dilutions of standard trans-resveratrol, ranging from 10-40 µg/mL, were prepared in ethanol. Each sample including the extract was sonicated for 15 minutes at 20˚C and syringe filtered using 0.45 µm filter prior to HPLC analysis (Agilent 1100 series, Agilent Technologies, Germany). Isocratic elution with methanol was done at flow rate of 1 ml/min; column (C-18, 150mm x 4.6mm, 5 µm), UV detector (307nm and 320 nm); runtime of 4 minutes and injection volume of 20 µL. The samples were chromatographed on a Phenomenex C18 column (250 mm × 4.6 mm, 5 µm) column. The mobile phase used was methanol: phosphate buffer (63:37%, v/v), (pH 6.8 adjusted with 0.5% (v/v) orthophosphoric acid solution in Milli-Q water,) (63:37%, v/v) filtered through a 0.22 µm nylon membrane and ultrasonically degassed prior to use. The mobile phase was delivered at a flow rate of 1.0 ml/min. The injection volume was 50 µLl. The eluate was monitored at 306 nm by an ultraviolet detector. Ttrans-Rresveratrol concentrations were calculated as concentration calculated through HPLC divided by g/ml (whole plant extract preparation) to give final concentration as µg/g of peanut on a dry weight basis. All experiments were performed in independent quadruplicates. All values are expressed as mean ± standard deviation with n=4.

~~*Arachis hypogaea L.* Growth~~

~~After sowing the seeds, emergence of seedlings occurred in 20 days . The plant that grew normally, after two months it was taken for further treatment.~~

E. Yeast Growth

It was used a Baker Yeast collected from shop. For activation, 1g table sugar was dissolved in 100ml warm water in a glass beaker.and 0.5 g yeast added in vessel and kept for overnight. Next day ~~in morning when~~it was observed, the fluffy rising growth of yeast ~~found~~ with foam~~roth~~.

Composition of PD Agar media

Potato extract 0.4g~~m~~, Dextrose 2.0 g~~m~~ and Agar 1.5 g~~m~~ pH-5.6

F. Gene Expression Profiling

1. Control : Wild *Saccharomyces cerevisiae* grown without any stress
2. Test 1 : *S. cerevisiae* grown with 2ml Aspergillus *niger* infected peanut plant extract
3. Test 2 : *S. ~~accharomyces~~ cerevisiae* grown with pure 100 µl (HPLC std.) trans-resveratrol.
4. Test 3 : *S. ~~accharomyces~~ cerevisiae* grown with 2ml wild peanut plant extract.

No. of primers used : 1. Beta actin and 2. Sir2

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Wild type Yeast cultures and Test samples (above mentioned in sample info) were grown overnight at 30^0 C in a tube rotator to ~2x10 7 cells/ml in 5ml of YPD. Samples from centrifugal elutriation size selection were immediately used from elutriation. Cell size and concentration were measured with a Z2 Coulter Counter. Total RNA Yeast lifespan variation correlates with cell growth and SIR2 expression was harvested from wild type and test cells using Tri-extract RNA isolation method (GBiosciences). After checking the isolated RNA concentration and purity with spectrophotometer, the samples were diluted to 20 ng/ul.

Synthesis of cDNA: ~~Added~~ Total RNA 5 µl, Oligo(dT)16 2µl, Rnase free dd water 7.5µl~~.~~ were Heated at 70^0 C for 5 minutes and ~~place~~ the tube was immediately placed on ice for 2 min. It was ~~Vortex~~ briefly mixed with a vortex and t~~een added~~ the following components~~.~~ were added ~~Added~~: 5xM-MLV Buffer 4µl, dNTPs 1µl, RNAsin 0.5µl, M-MLV 1µl and incubated at 42^oC for 60 min. ~~Heat t~~The sample was heated to 95^oC to inactivate enzyme~~.~~ and ~~c~~Cooled ~~the sample~~ on ice for downstream experiments or store at -20^0C.

Primer Sequences of Sir2 F-ggcagtgtcagcagcttcag, Sir2 R-gggcuencegtctctggtttcaaaa, primer sequence ACT1 F- tcgttccaatttacgctggtt, ACT1R-cggccaaatcgattctcaa. Normal PCR reaction carried out separately to ensure reaction prior to real time PCR with the following cocktail and program~~.~~: PCR master mix 25µl, Primer F (10uM) 1µl, Primer R 1µlcDNA 5µl, ddwater 18µl~~.~~ ~~PCR Program:~~at 95^0C for 3 minutes, 95^0C for 20 seconds, 50^0C (Act1)/52 ^0C (Sir2) for 20 seconds, 72^0C for 40 seconds for 40x, 72^0C for 3 minutes, 4^0C for 10 minutes

Real Time PCR : The ~~C~~cocktail~~:~~ was prepared with Syber green Master mix 25.0 µl, Primer F 2.0 µl, Primer R 2.0 µl, cDNA 5.0 µl, dH_2O 16.0 µl. The Real Time PCR Program was **:** Activation 95^0C, 2 minutes 1 cycle, Denaturation: 95^0C, 5 minutes, 40 Cycles (95^0C for 10 seconds, 52 for 20 seconds, 72 for 10 seconds)

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Real time PCR reactions were carried out using Kappa SYBR Fast Master Mix (2X) with ROX reference dye. Gene Expression primers~~–~~ for budding yeast SIR2 and ACT1 genes primers were obtained from GCC Biotech. Gene expression analysis of the real-time data was conducted with ABI Prism 7000 SDS software as well as Excel. Relative gene expression was calculated using the $2^{-\Delta\Delta CT}$ comparative C_T method.

Preparation of Yeast Growth~~-~~ Media and studying Replicative Life Span ~~through Spectrophotometer~~

Yeast startup in sugar solution was ~~S~~streaked on PD agar plates (Potato extract 0.4g~~m~~, Dextrose 2.0 g~~m~~ and Agar 1.5 g~~m~~, pH-5.6) . ~~Incubate t~~The cells were incubated at 36℃ for 48 hours, when single colonies appeared. ~~Picked s~~Single colonies were picked and inoculated into 50 mL of Potato Dextrose liquid medium in conical flask (Potato extract 0.4g~~m~~, Dextrose 2.0 g~~m~~, pH-5.6). ~~Grown c~~Cultures were grown overnight at 36℃ for 48 hours while maintaining constant agitation using a orbital shaker incubator~~,~~ (Thermotech).

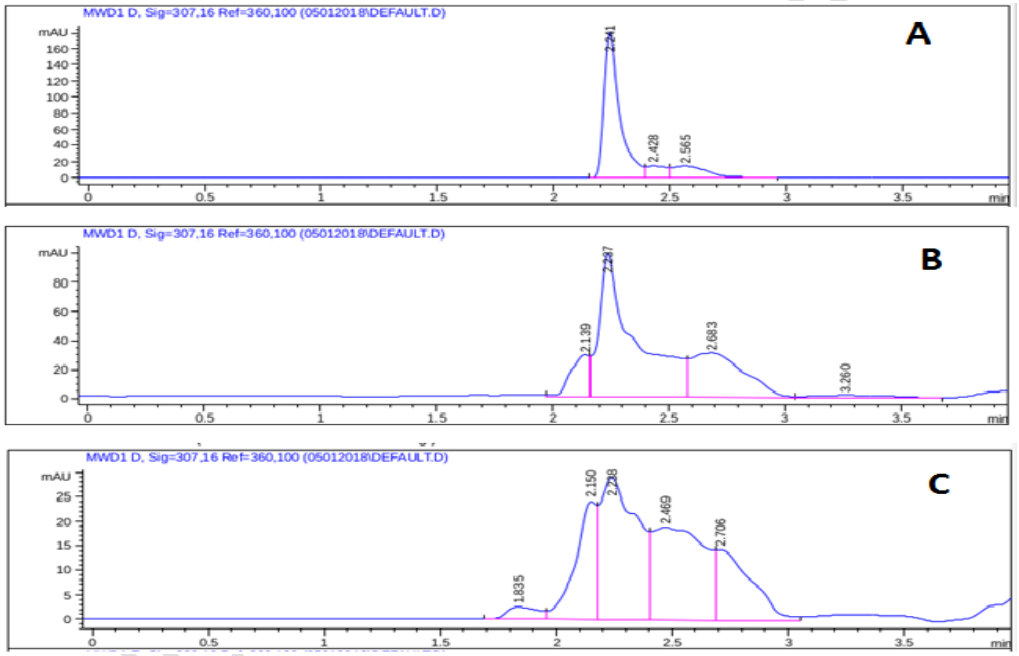
~~Distributed~~ 5 mL of 48 hours old culture were distributed in 10 test tubes, the first test tube contains only culture and marked as control, then triplicate of standard, normal and infected peanut plant extract were prepated for biological replication of cells. ~~Maintain the c~~Cultures were mantained at 36℃ with constant agitation on Orbital shaker incubator for the entire experiment for two weeks. Absorbance was measured ~~taken~~ at 600nm by spectrophotometer (Thermo Helios).

RESULTS

Analysis of trans-resveratrol through HPLC

The extracts were analysed by reversed phase high-performance liquid chromatography (RP-HPLC) using ~~mobile phase,~~ methanol and phosphate buffer (63:37%, v/v) as mobile phase. The mobile phase was chosen for its good baseline resolution and suitable analysis duration. The optimal detection wavelength was found to be at 307 nm, as determined by the maximum peak of resveratrol standard. This wavelength is reported in several studies and the retention time is ≈2.24 minutes. The limit of detection (LOD) is 1 µg/ml while the limit of quantification (LOQ) is 5 µg/ml.

Figure 1. HPLC chromatograms at 307 nm A: trans-resveratrol standard; B. Infected plant extract (Aspergillus niger infected peanut plant); C: Normal peanut plant extract.



Concentration of trans-resveratrol

The values of resveratrol in control and infected plants are~~is~~ given in Table 1. The control plants exhibit 0.82±0.03 mg/g of resveratrol while the infected plants show 2.7 fold increase in resveratrol production as 2.24±0.3 mg/g of dried plant residue (Figure 2), the formula used in calculation was: *Response Factor = (mAU of known std./ concentration of std)*, then *Ceoncentration in sample = (mAU of sample/Response Factor)*.

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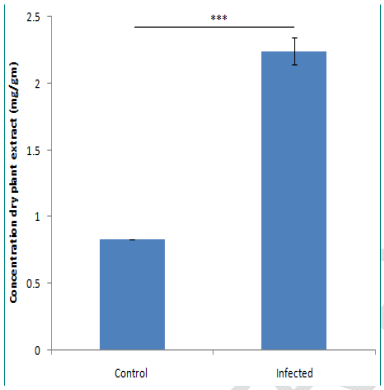
Table 1–: Concentration of trans-resveratrol obtained in control and *Aspergillus niger* infected whole plant extracts. Four plants were taken in each group. All the values are in mean ± std.dev. (n=4)

Peanut plant	Retention time (minutes)	Peak Area (mAU)	Response Factor	Concentration (ug/ml)	Concentration of dry plant extract (mg/g)
Normal peanut plant	2.2375±0.005	337.6675±14.65	40.75	8.285±0.35	0.83±0.036
Infected peanut plant	2.2425±0.005	914.1±131.096	40.75	22.4325±3.21	2.245±0.32

Figure 2: Relative concentration of trans-resveratrol in control and *Aspergillus niger* infected whole plant extracts



*** means p<0.001

Yeast culture preparation and microscopic examination

Yeast startup inwith sugar solution was streaked in PDA media Petri plate and a ; single colony was isolated isolation of yeast in PDA media petri plate then yeast waswas and examined through microscope at 100X

Figure 3: a. Yeast growth in sugar, b. Single colony isolation, c. Microscopic examination of yeast cell at 100x

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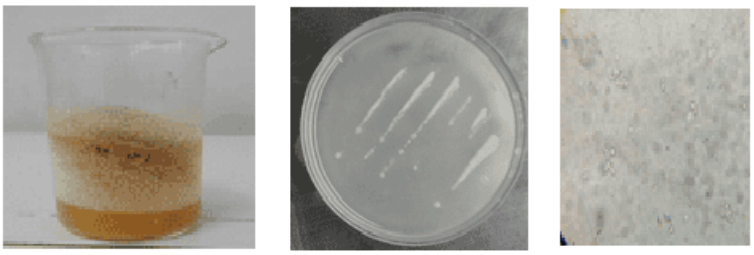
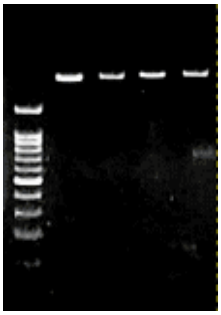


a **b.** **c.**

Agarose Gel Electrophoresis Image of RNA

Gel was prepared with 1% w/v Agarose in 1X Tris Acetic Acid Ethylene Diamine Tetra Acetic Acid (TAE) buffer and run at 100^{0}C. Gel was illuminated at 302 nm UV trans-illuminator to obtain image. (Figure 4)

Figure 4: From left side, well No.1 contains Molecular Ladder, well No.2-wild yeast RNA, well No. 3-Test1 RNA, well No.4-Test2 RNA, well No.5-Test3 RNA.



***A*mplification Curve**

Real time PCR~~pcr~~ amplification plots for ACT1 gene of 16 reactions vials are shown in figure 8~~.~~ and SIR2 gene in figure 5.

Figure 5: Amplification curve of ACT1 primer, 16 curves (each Ct value i~~s~~n given in table 2)

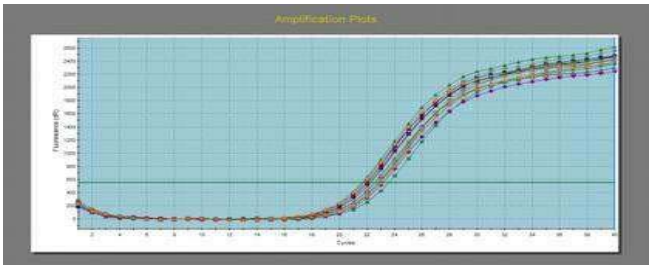
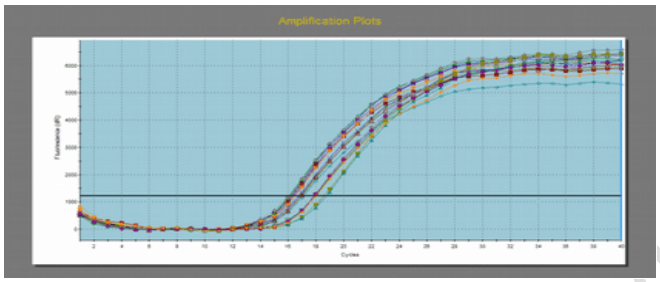


Figure 6: Amplification Plots of SIR2 primer, 16 curves (each Ct value in given in table 3)



Delta ~~Ct~~ C_T value with ACT1 gene of control and test appears in Fig.5

Table2:1st Column:wells designation, 2nd column: Primer used (ACT1), 3rd column sample information (each sample has been used 4 times to increase the accuracy), 4th column: Threshold Cycle value (C_T) 5th Column: Mean value of all 4 value for each sample, 6th column: Delta C_T value

Well	Primer	Sample		C_T(dR)	Mean C_T	ΔC_T
A1	ACT1	Test1	1	21.75	21.65	-1.482
A2			2	21.88		
A3			3	21.45		
A4			4	21.52		
A5		Test2	1	22.53	22.11	-1.025
A6			2	22.72		
A7			3	21.58		
A8			4	21.60		
B1		Test3	1	22.06	22.45	-0.685
B2			2	22.36		
B3			3	22.73		
B4			4	22.64		
B5		Control	1	23.38	23.13	
B6			2	23.38		
B7			3	22.94		
B8			4	22.86		

Delta C_T value with SIR2 gene of control and test appear in Fig.6

Table 3:1st Column:wells designation, 2nd column: Primer used (Sir2), 3rd column sample information (each sample has been used 4 times to increase the accuracy), 4th column: Threshold Cycle value (C_T) 5th Column: Mean value of all 4 value for each sample, 6th column: Delta C_T value

Well	Primer	Sample		C_T(dR)	Mean C_T	ΔC_T
C1	SIR2	Test1	1	16.26	16.22	-2.125
C2			2	16.37		
C3			3	16.15		

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C4			4	16.08		
C5		Test2	1	16.98	16.80	-1.540
C6			2	17.14		
C7			3	16.48		
C8			4	16.60		
D1		Test3	1	16.82	17.42	-0.923
D2			2	16.96		
D3			3	17.96		
D4			4	17.93		
D5		Control	1	18.63	18.34	
D6			2	18.79		
D7			3	17.92		
D8			4	18.02		

Fold change of Sir2 genes with tests are tabulated in Table 2

Table 4: Fold change of Sir2 gene in control and with yeast culture having trans-resveratrol extracted from peanut plant and standard trans-resveratrol

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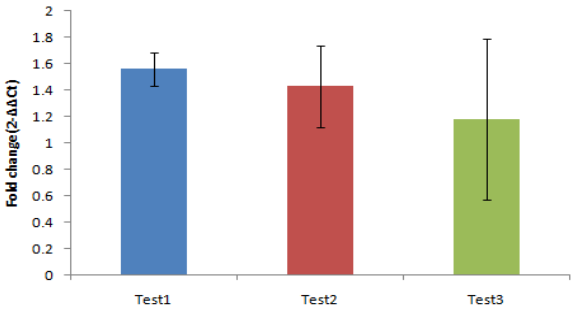
Sample	ΔCt [Sir2]	ΔCt [Act1]	ΔCt [ΔCt [Sir2]-ΔCt [Act1]]	Fold Change [Sir2]
Test1	-2.1250	-1.4825	-0.6425	1.56
Test2	-1.5400	-1.0250	-0.5150	1.43
Test3	-0.9225	-0.6850	-0.2375	1.18

Relative gene expression of all 3 samples is in bar graph (Figure XX)

Figure 7: Relative comparison of Sir2 gene expression in 3 samples



Replicative Life Span

Figure 8: 1. Yeast pPlate streaking for single colonly isolation, 2. Incubation of yeast culture in triplicates

1. Yeast Streak plate

2. Yeast Culture in triplicates

Table 5: 600 nm Aabsorbance column 1st row Standard test tube contains only media and yeast culture absorbance is 0.138, 2nd row Normal plant extract containing media and yeast culture shows slight increase in absorbance 0.155, 3rd row Infected plant extract containg media and yeast culture shows enhanced absorbance 0.522.

Sample Name	Spectrophotometer Absorbance at 600nm (triplicate)			Mean Absorbance	Standard deviation	Absorbance
1. Standard (Media + Yeast Culture)	0.144	0.138	0.132	0.138	0.006	0.138±0.006
2. Normal Plant extract + Media + Yeast Culture	0.154	0.158	0.152	0.155	0.003	0.155±0.003
3. Aspergillus niger infected peanut plant extract + Media +Yeast culture	0.530	0.520	0.515	0.522	0.008	0.522±0.008

~~CONCLUSION AND~~ DISCUSSION

In conclusion, biotic stress in the form of *Aspergillus niger* and abiotic stress in the form of wounding results in an increase in resveratrol content of experimental peanut plants. Thus this study reports a combination of elicitation methods on peanut plant, an important agricultural crop in India and a promising functional food, to develop as a source of the bioactive compound resveratrol.

Sir2 gene expression produces sirtuin enzyme. Sirtuin enzyme is group of Histone Deacetylate Transferase enzyme that removes acetyl (-) group from lysine (+) amino acid situated on Histone. Thus negative ion DNA shows affinity towards + charge and get wrapped along Histone. Due to wrapping, RNA polymerase will not find site for transcription, hence, this promotes gene silencing.

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Comment [SBB16]: It is a repetition of the introduction but not a discussion of the results obtained in this work.

~~In all three test conditions~~*Saccharomyces cerevisiae* sir2 gene expression increased in the three test conditions ~~*Saccharomyces cerevisiae.*~~ This result confirms the hypothesis that trans-resveratrol actually enhance expression of sirtuin enzyme which is a class of histone transferase enzyme. Due to over activation of histone transferase enzyme, process of gene silencing occurs. When organism aged, accumulation of multiple genes or noise genes stops~~halt~~ the processes of cell and ~~that~~ affects the rate of cell division ~~also~~. Enhanced sirtuin enzyme (product of sir2 gene) accelerates gene silencing process of undesired gene and hence cell reverses its age.

The ~~R~~replicative ~~L~~life ~~S~~span of Yeast cell increased due to the presence of enhanced trans-resveratrol peanut plant extract. It related epigentics change in sir2 gene. All results indicated reverse aging in yeast.

Indian groundnuts are available in Bold or Runner, Java or Spanish and Red Natal varieties. Peanut being a good source of protein and fiber, also contains resveratrol which is slowly gaining attention in the Indian market as a health supplement.

CONCLUSION

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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Comment [SBB17]: It has been said many times across the manuscript, so it is not necessary to say it each time that the enzyme is named.

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Comment [SBB18]: This sentence is not understandable

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Comment [SBB19]: If the article have more than six authors, list the first six authors followed by et al.

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