

Original Research Article

<https://doi.org/10.20546/ijcmas.2018.704.399>

Influence of Seed Priming on Biochemical Changes in Fresh and Aged Seeds of Sunflower (*Helianthus annuus* L.) Hybrid KBSH-53

B. Manjunatha*, B.C. Channakeshava, Y.G. Shadakshari, K. Bhanuprakash, K.R. Sreeramulu and P. Balakrishna

Department of Seed Science and Technology, College of Agriculture, University of Agricultural Sciences, Bangalore-560065, Karnataka, India

*Corresponding author

ABSTRACT

Keywords

Sunflower (*Helianthus annuus* L.), Biochemical

Article Info

Accepted:

28 March 2018

Available Online:

10 April 2018

Priming on biochemical changes was tested on two seed lots L₁: Fresh seeds (> 91 % Germination) and L₂: Aged seeds (< 70 % Germination). The seeds were treated with priming chemicals 12 hours of soaking viz., C₁: Control, C₂: Hydropriming, C₃: KH₂PO₄ (1 %), C₄: GA₃ (400 ppm), C₅: NaCl (1 %), C₆: KCl (2 %), C₇: KNO₃ (2 %), C₈: CaCl₂.2H₂O (2 %), C₉: PEG (-1Mpa), C₁₀: K₂HPO₄ (1 %). Among the various seed priming treatments KH₂PO₄ (1 %) is the suitable priming chemical to improve the marginal quality sunflower seed lots viz., higher total dehydrogenase activity (OD@ A480nm) (0.909), α -Amylase activity (mg maltose liberated min⁻¹ g⁻¹) (0.530), Peroxidase activity (ΔA436 nm min⁻¹ gram⁻¹) (0.732) and lower electrical conductivity (1.722 dSm⁻¹), as compared to control (0.568, 0.258 and 1.722 dSm⁻¹ respectively). Priming treatments are more effective in marginal seed lots compared to fresh seeds.

Introduction

Sunflower (*Helianthus annuus* L.) belongs to the family Astreaceae and it is one of the world's most important sources of vegetable oil. The native of sunflower is reported to be southern parts of USA and Mexico. Sunflower ranks third, next to groundnut and soybean in total production. In world it is cultivated in an area of 25.56 million hectares with an annual production of 40.64 million tonnes with productivity of 1590 kg ha⁻¹ during 2015 (Anon., 2016).

Sunflower was introduced to India during 1969 as a supplement to traditional oilseed

crops to bridge the gap of recurring edible oil shortage in the country. The commercial cultivation of sunflower was started in India during 1972-73 with an introduction of Russian varieties from USSR and Canada. Now, the crop is well adopted because of attributes such as short duration, photoperiod insensitivity, adoptability to wide range of soil and climatic conditions, drought tolerance, higher seed multiplication ratio (1:50) having high percentage of edible oil (45-50 %), which contains polyunsaturated fatty acid (PUFA). In recent years, India has emerged as second major sunflower producing country in Asia after China. In India, it is being grown in an area of 0.52 million hectares with annual

production of 4.2 million tonnes having a productivity of 750 kg ha⁻¹ (Anon., 2014).

Priming in its traditional sense, soaking of seeds in water before sowing, has been the experience of farmers in India in an attempt to improve crop stand establishment but the practice was without the knowledge of the safe limit of soaking duration (Harris 1996). Moreover, Harris *et al.*, (1999) promoted a low cost, low risk technology called 'on farm seed priming' that would be appropriate for all farmers, irrespective of their socio economic status. On-farm seed priming involves soaking the seed in water, surface drying and sowing the same day. The rationale is that sowing soaked seed decrease the time needed for germination and allow the seedling to escape deteriorating soil physical conditions. According to Khan (1992), osmotic conditioning in its modern sense, aims to reduce the time of seedling emergence, as well as synchronize and improve the germination percentage, by subjecting the seeds to a certain period of imbibition using osmotic solutions. The seeds normally begin water uptake on contact with this solution and stop the process as soon as they become balanced with the water potential of the solution.

Seed priming is basically a pre-sowing seed treatment. However, osmoprimed seeds may be dried back to their initial moisture content and stored for variable periods of time depending on the species. Primed and dried seeds normally have a more rapid and uniform germination when subsequently re-hydrated, especially under adverse environmental conditions (Bradford, 1986). The mechanism of seed drying after chemical priming is known as the hydration-dehydration process or dry back and is used to reduce the degree of moisture in seeds to levels compatible with storage and maintaining the beneficial effects of the treatment, without quality loss caused by rapid seed deterioration. The advantage of

seed priming is reducing the germination time and improving emergence in field and laboratory conditions. However, few detailed studies have been reported on the performance of somatically treated seeds under field conditions. (Kumar *et al.*, 1996) reported that priming of aged seeds of okra resulted in good germination and better crop establishment in the field trials. The benefits of seed priming was in terms of faster emergence, more and uniform stands, less need to re-sow, more vigorous plants, drought tolerance, earlier flowering, maturity and higher seed yield.

Beneficial effect of these seed priming treatments were reflected in greater cellular membrane integrity, counter action of lipid peroxidation and free radical chain reaction often found to be directly correlated with the maintenance of viability and reduced moisture uptake by hydrated dehydrated seeds (Dollypan and Basu, 1985), antipathogenic effects, repair of biochemical lesions by cellular enzymatic repair system and metabolic removal of toxic substances and counteraction of free radical and lipid peroxidation reactions (Rudrapal and Basu, 1982).

Invigoration of seeds is accompanied by certain biochemical changes in seed. Therefore, the present investigation was carried out to study the Influence of seed priming on biochemical changes in fresh and aged seeds of sunflower hybrid KBSH-53.

Materials and Methods

The laboratory experiment was conducted in 2016, at Department of Seed Science and Technology, University of Agricultural Sciences, Bengaluru. Treatments consists of sunflower hybrid KBSH-53 seed lots: L₁: Fresh seeds (> 91 % Germination), L₂: 10 months aged seeds (< 70 % Germination) and priming Treatments: C₁: Control, C₂: Hydro

priming, C₃: KH₂PO₄ (1 %), C₄: GA₃ (400 ppm) C₅: NaCl (1 %), C₆: KCl (2 %), C₇: KNO₃ (2 %), C₈: CaCl₂.2H₂O (2 %), C₉: PEG (-1 Mpa) and C₁₀:K₂HPO₄ (1 %), seeds are soaked for 12 hours in solutions. The experiment was carried out in factorial completely randomized design in three replications and observations on various seed quality parameters were recorded.

Electrical conductivity of seed leachate (dSm⁻¹)

Twenty five seeds of three replications were taken randomly from each treatment in a beaker. Then the seeds were soaked in 25 ml of distilled water for 24 hours at 25 ± 1 °C. The steeped water from soaked seeds was collected and the electrical conductivity (EC) of seed the leachate was measured in digital conductivity meter (Model: Systronic conductivity meter 306).

After subtracting the EC of the distilled water from the value obtained from the seed leachate, the actual EC was measured and expressed in dSm⁻¹.

Total dehydrogenase activity (TDH)

Ten sunflower seeds from each treatment were preconditioned by soaking in water for 24 hours. Then, seed coat was removed and soaked in 2 ml of 0.5 per cent tetrazolium solution for four hours at 25 ± 1 °C in dark and then washed thoroughly with distilled water. The red colour (Formazan) developed was eluted from the stained embryos by soaking in 5ml of 2 Methoxy ethanol in screw capped vials complete elution.

The extract was decanted and the colour intensity was measured in spectrophotometer (model SL 171) at 480 nm with suitable blank. The TDH was expressed in terms of absorbance value (Perl *et al.*, 1983)

α-Amylase activity (mg maltose liberated min⁻¹ gram⁻¹)

Reagents prepared

0.1M Phosphate buffer (pH 7.0)

1.0 % Soluble starch solution: 1.0 g of soluble starch was dissolved in 100 ml of warm 0.1 M sodium acetate buffer (pH 4.7)

Dinitrosalicylicacid (DNS) reagent: 1.0 g of 3, 5-dinitrosalicylicacid, 200 mg of crystalline phenol and 50 mg of sodium sulphite was dissolved in 100 ml of 1 per cent NaOH solution by stirring. Reagent was stored in a stoppered bottle at 40 °C.

40 % Rochelle salt solution (potassium sodium tartrate): 40 g of salt was dissolved in 100 ml of water.

Standard maltose solution: 50 mg of maltose was dissolved in water and the volume was made up to 50 ml in a volumetric flask (1 mg/ml) and stored in refrigerator until the assay.

Preparation of seed powder and enzyme extraction

Seed powder was prepared from different ageing treatment seeds. Immediately after ageing, seeds were washed thoroughly and excess moisture was removed by blotting with tissue paper. Then 0.8 g seeds were frozen in liquid nitrogen and ground with the help of pestle and mortar. While grinding seed itself, 1.0 ml of 0.1 M phosphate buffer (pH 7.0) was added and the ground material was transferred to eppendorf tubes and kept at 4 °C overnight and then transferred to -20 °C.

The slurry was centrifuged at 10,000 rpm for 15 minutes at 4 °C and the supernatant was used for α -amylase assay.

Enzyme assay

The α -amylase assay was carried out according to the method of Bernfeld (1955) with slight modification. The enzyme assay of sample was carried out along with blank and control for each sample. For sample analysis 0.1 ml of enzyme extract was taken in a cleaned test tube and 250 μ l of 1 per cent soluble starch was added and incubated for 15 min. To this 500 μ l of DNS reagent was added to stop the reaction and heated over water bath for 5 min and then cooled under running tap water after this, 250 μ l of 40 % sodium potassium tartrate was added. Final volume of the reaction mixture was made to 5 ml by adding 3.9 ml of water. Absorbance was read at 560 nm. A control was prepared for each sample similar to that of sample but the reaction was terminated at zero time. Similarly blank was prepared for each sample by omitting starch. Standard curve was prepared by using maltose (0 to 100 μ g). One unit of enzymatic activity is defined as one mg of maltose liberated/hour under the standard assay conditions and specific activity as mg maltose liberated/min/gram seed.

Peroxidase activity ($\Delta A_{436} \text{ nm min}^{-1} \text{ gram}^{-1}$)

Enzyme extraction

One gram of sunflower seeds subjected to different ageing treatments were extracted in 1ml of 0.1 M Phosphate buffer with pH 7.0 by grinding with a pre cooled pestle and mortar.

The slurry was transferred to eppendorf tubes and kept at 4 °C for 4 hours for enzyme extraction and then tubes are transferred to 20 °C. The homogenate was centrifuged at 10000 rpm at 4 °C for 15 minutes. The supernatant was used as enzyme source. The enzyme extract was stored in ice box till the assay is carried out.

Preparation of reagents

Phosphate buffer 0.1 M (pH 7.0)

Guaiacol solution (20 mM): 242 μ l guaiacol was added to distil water and the volume was made to 100 ml. It can be stored in frozen condition for many months.

Hydrogen peroxide solution (0.042 %) (12.3 mM): 125 μ l of 30 per cent hydrogen peroxide was added to distilled water and volume was made to 100 ml. It should be prepared at the time of use. Absorbance of 12.3 mM H₂O₂ was adjusted to 0.4 by adding 20 ml of water to it, before using the solution for assay.

Estimation of peroxidase activity

The enzyme assay was carried out as per the procedure of Sadasivam and Manickam (2008). The reaction mixture was prepared in cuvette by adding 2 ml of 0.1 M phosphate buffer of pH 7.0, Guaiacol-200 μ l and 12.3 mM H₂O₂-200 μ l. Brought the mixture to 25 °C and then placed the cuvette in the Spectrophotometer set at 436 nm. Then, add 100 μ l of enzyme extract mix it properly with pipette tip, immediately start the stopwatch. Read the initial absorbance at 436 nm and note increase the absorbance for 3 minutes at an interval of 30 seconds by using enzyme kinetics. Water is used as blank during the assay period and enzyme activity was expressed in terms of change in absorbance per minute per gram of seed.

Results and Discussion

The data on electrical conductivity (EC) as influenced by the seed lots and priming treatments are presented in table 1. The electrical conductivity exhibited significant variations due to seed lots and priming treatments and their interactions. Between the seed lots, electrical conductivity was lower in

L₁ (0.291 dSm⁻¹) and it was higher in L₂ (2.861 dSm⁻¹). Among the priming treatments, lowest (1.225 dSm⁻¹) electrical conductivity was recorded in C₃ followed by C₄ and C₁₀ (1.242 and 1.442 dSm⁻¹ respectively). However, highest electrical conductivity was recorded in C₁ (1.722 dSm⁻¹). Electrical conductivity differed significantly due to L×C. Among the interactions, lower electrical conductivity was recorded in L₁C₃ (0.117 dSm⁻¹) followed by L₁C₄ and L₁C₉ (0.130 dSm⁻¹). However, higher electrical conductivity was observed in L₂C₁ (3.237 dSm⁻¹).

The EC value was significantly lower (0.291 dSm⁻¹) in high vigour seeds compared to higher value (2.861 dSm⁻¹) recorded in low vigour seeds. High vigour seeds primed with KH₂PO₄ had registered lower EC (1.225 dSm⁻¹) as against highest (1.722 dSm⁻¹) noticed in control. The EC value is used as an index of loss of cell membrane integrity and it is negatively correlated with the seed quality attributes. The distinct reduction in EC of seed leachate may be due to restoration of membrane integrity upon priming so that the leaching of electrolytes might be controlled in primed seeds (Sung and Chang, 1993). The similar results were also recorded by Sowmya (2011) in cucumber and Radha (2013) in maize.

The data on total dehydrogenase activity (TDH) as influenced by the seed lots and priming treatments are presented in table 1. The total dehydrogenase activity exhibited significant variations due to seed lots and priming treatments and their interactions. Between the seed lots, total dehydrogenase activity was higher in L₁ (0.859) and it was lower in L₂ (0.716). Among the priming treatments, highest total dehydrogenase activity was recorded in C₃ (0.909) followed by C₄ and C₉ (0.904 and 0.896 respectively). However, lowest total dehydrogenase activity was recorded in C₁ (0.568). Total

dehydrogenase activity differed significantly due to L×C. Among the interactions, higher total dehydrogenase activity was recorded in L₁C₃ (0.976) and L₁C₄ followed by L₁C₉ and L₁C₁₀ (0.950 and 0.943 respectively). However, lower (0.427) total dehydrogenase activity was observed in L₂C₁. The TDH was higher (0.859) in high vigour seeds but it was extremely lower (0.716) in low vigour seeds.

Among the priming treatments, higher total dehydrogenase activity (0.909) was recorded in KH₂PO₄ (primed seeds (C₃) and it was lower (0.568) in control (T₁). Increased TDH activity might be an index of increased cellular biosynthetic activities like DNA and RNA synthesis that in turn indicate the higher protein and energy production necessary for germination and seedling emergence (Osborne *et al.*, 1980). Priming increased enzyme activity as well as counteracted the effects of lipid peroxidation. Saha *et al.*, (1990) showed that priming caused increased α-Amylase and dehydrogenase activity in aged soybean seeds compared to unprimed seeds while decreased lipid peroxidation. The results are in line with Sowmya (2011) in cucumber and Radha (2013) in maize.

The data on α-Amylase activity as influenced by the seed lots and priming treatments are presented in table 2. The α-Amylase activity exhibited significant variations due to seed lots and priming treatments and their interactions. Between the seed lots, α-Amylase activity was higher in L₁ (0.507 mg maltose liberated min⁻¹ g⁻¹) and it was lower in L₂ (0.263). Among the priming treatments, highest α-Amylase activity was recorded in C₃ (0.530 mg maltose liberated min⁻¹ g⁻¹) followed by C₄ and C₉ (0.515 and 0.490 mg maltose liberated min⁻¹ g⁻¹ respectively). However, lowest α-Amylase activity was recorded in C₁ (0.258 mg maltose liberated min⁻¹ g⁻¹). α-Amylase activity differed significantly due to L×C.

Table.1 Influence of seed lots and priming treatments on electrical conductivity (dSm⁻¹) and dehydrogenase activity (A480) of sunflower hybrid KBSH-53

Treatments	Electrical conductivity (dSm ⁻¹)			Dehydrogenase activity (A480)		
	L ₁	L ₂	Mean	L ₁	L ₂	Mean
C ₁	0.207	3.237	1.722	L ₁	L ₂	0.568
C ₂	0.190	3.147	1.668	0.710	0.427	0.675
C ₃	0.117	2.333	1.225	0.740	0.610	0.909
C ₄	0.130	2.353	1.242	0.976	0.842	0.904
C ₅	0.167	3.153	1.660	0.976	0.833	0.715
C ₆	0.163	3.040	1.602	0.780	0.650	0.730
C ₇	0.157	2.857	1.507	0.790	0.670	0.792
C ₈	0.140	2.743	1.442	0.850	0.733	0.820
C ₉	0.130	2.823	1.477	0.877	0.763	0.896
C ₁₀	1.507	2.920	2.213	0.950	0.843	0.867
Mean	0.291	2.861	CV (%) 3.58	0.943	0.791	CV (%) 3.02
	S.Em±	CD(P=0.01)		0.859	0.716	
L	0.008	0.024		S.Em±	CD(P=0.01)	
C	0.019	0.054		0.003	0.011	
LC	0.026	0.077		0.008	0.024	

Lots:

L₁: Fresh seeds (> 91 % Germination)
L₂: 10 months aged seeds (< 70 % Germination)

Priming Treatments:

C₁: Control
C₂: Hydropriming
C₃: KH₂PO₄ (1%)
C₄: GA₃ (400 ppm)
C₅: NaCl (1 %)
C₆: KCl (2 %)
C₇: KNO₃ (2 %)
C₈: CaCl₂.2H₂O (2%)

C₉: PEG (-1 Mpa)
C₁₀: K₂HPO₄ (1 %)

Table.2 Influence of seed lots and priming treatments on α – amylase (mg maltose liberated $\text{min}^{-1} \text{gram}^{-1}$) and peroxidase ($\Delta\text{A436 nm min}^{-1} \text{gram}^{-1}$) of sunflower hybrid KBSH-53

Treatments	α -Amylase (mg maltose liberated $\text{min}^{-1} \text{gram}^{-1}$)			Peroxidase ($\Delta\text{A436 nm min}^{-1} \text{gram}^{-1}$)		
	L ₁	L ₂	Mean	L ₁	L ₂	Mean
C ₁	0.323	0.193	0.258	0.687	0.307	0.497
C ₂	0.337	0.200	0.268	0.713	0.333	0.523
C ₃	0.723	0.337	0.530	0.927	0.537	0.732
C ₄	0.710	0.320	0.515	0.910	0.540	0.725
C ₅	0.340	0.247	0.293	0.730	0.530	0.630
C ₆	0.350	0.243	0.297	0.757	0.450	0.603
C ₇	0.417	0.270	0.343	0.790	0.490	0.640
C ₈	0.537	0.273	0.405	0.827	0.521	0.674
C ₉	0.693	0.287	0.490	0.870	0.591	0.731
C ₁₀	0.640	0.257	0.448	0.853	0.573	0.713
Mean	0.507	0.263	CV (%) 4.02	0.806	0.487	CV (%) 4.56
	S.Em±	CD(P=0.01)		S.Em±	CD(P=0.01)	
L	0.008	0.024		0.004	0.012	
C	0.019	0.055		0.010	0.028	
LC	0.027	0.078		0.014	0.040	

Lots:

L₁: Fresh seeds (> 91 % Germination)
L₂: 10 months aged seeds (< 70 % Germination)

Priming Treatments:

C₁: Control
C₂: Hydropriming
C₃: KH₂PO₄ (1%)
C₄: GA₃ (400 ppm)
C₅: NaCl (1 %)
C₆: KCl (2 %)
C₇: KNO₃ (2 %)
C₈: CaCl₂.2H₂O (2%)

C₉: PEG (-1 Mpa)
C₁₀: K₂HPO₄ (1 %)

Among the interactions, higher α -Amylase activity was recorded in L₁C₃ followed by L₁C₄ (0.723 mg maltose liberated min⁻¹ g⁻¹) and L₁C₉ (0.710, and 0.693 mg maltose liberated min⁻¹ g⁻¹ respectively). However, lower α -Amylase activity was observed in L₂C₁ (0.193 mg maltose min⁻¹ g⁻¹). Priming increased enzyme activity as well as counteracted the effects of lipid peroxidation. Saha *et al.*, (1990) showed that priming caused increased α -Amylase and dehydrogenase activity in aged soybean seeds compared to unprimed seeds while decreased lipid peroxidation. The results are in line with Sowmya, (2011) in cucumber and Radha (2013) in maize.

The data on peroxidase activity as influenced by the seed lots and priming treatments are presented in table 2. The peroxidase activity exhibited significant variations due to seed lots and priming treatments and their interactions. Between the seed lots, peroxidase activity was higher in L₁ (0.806) and it was lower in L₂ (0.487). Among the priming treatments, highest peroxidase activity was recorded in C₃ (0.732) followed by C₄ and C₉ (0.725 and 0.731 respectively). However, lowest peroxidase activity was recorded in C₁ (0.497). Peroxidase activity differed significantly due to L×C. Among the interactions, higher peroxidase activity was recorded in L₁C₃ (0.927) followed by L₁C₄ and L₁C₉ (0.910 and 0.870 respectively). However, lower peroxidase activity was observed in L₂C₁ (0.307). Increased activity of free radicle scavenging enzymes such as superoxide dismutase, catalase, and peroxidase and glyoxysome enzymes such as isocitrate lyase and malate synthase upon priming. The results are in line with Sowmya (2011) in cucumber and Radha (2013) in maize.

It is evident from the present study seeds primed with KH₂PO₄ (1 %) shows lower

electrical conductivity of seed leachates (EC), higher dehydrogenase activity, α -Amylase activity and Peroxidase activity where, as seeds without priming shows higher EC, lower dehydrogenase activity, α -Amylase activity and Peroxidase activity. The correlation of biochemical parameters correlated with the vigour and viability of sunflower seeds showed that the electrical conductivity of seed leachates (EC) increases, vigour and viability of sunflower seeds decreases and high dehydrogenase activity was found to favour for invigouration of seeds.

References

- Anonymous, 2014, Production Year Book, Food and Agricultural Organization, Rome.
- Anonymous, 2016, Database on oilseed area, production and productivity, DOR, Directorate of Oilseeds Research, Rajendranagar, Hyderabad.
- Bernfeld, P., 1955, Amylases- V and E In: Methods in Enzymology, Academic Press, New York, pp.149-155.
- Bradford, K. J., 1986, Manipulation of seeds water relations via osmotic priming to improve germination under stress conditions. *Hort. Sci.*, 59(2): 672-676.
- Dollypan and Basu, R. N., 1985, Mid-storage and pre-sowing seed treatments for lettuce and carrot. *Scientia Hort.*, 33(6): 1026-1027.
- Harris, D., 1996, Effects of manure, genotype, seed priming, depth and date of sowing on the emergence and early growth of *Sorghum bicolor* (L.) Moench in semi-arid Botswana. *Soil Tillage Res.*, 40:73-88.
- Harris, D., Joshi, A., Khan, P. A., Gothkar, P. and Sodhi, P. S., 1999, On-farm seed priming in Semi-arid agriculture: development and evaluation in maize, rice and chickpea in India using

- participatory methods, *Exp. Agric*, 35: 15-29.
- Khan, A. A., 1992, Pre-plant physiological conditioning. *Hort. Rew.*, 13: 131-181.
- Kumar, S., Singh, P., Katiyar, R. P., Vaish C. P. and Khan, A. A., 1996, Beneficial effect of some plant growth regulators on aged seeds of okra (*Abelmoschus esculentus* (L.) Moench.) under field conditions. *Not Sci. Biol*, 21(2): 34-37.
- Osborne, D. J., Sharon, and Benishai, R., 1980, Studies on DNA integrity and DNA repairing germinating embryos of rye (*Secale cereal*). *Israel. J. Bot*, 29:259-272.
- Perl, M., Luria, I.G and Gelmand, H., 1983, Biochemical changes in sorghum seeds affected by accelerated ageing. *J. Expt. Bot.*, 29:497-509.
- Radha, B. N. 2013, Studies on influence of provenances, seed priming and seed storage in hybrid maize (*Zea mays* L.). *Ph.D. Thesis*. University of Agricultural sciences, Bangalore.
- Rudrapal, A. B. and Basu, R. N., 1982, Use of chlorine and bromine in controlling mustard seed deterioration. *Seed Res.*, 9: 188-191.
- Sadasivam, S. and Manickam, 2008, Biochemical methods, Chapter IV: *Enzymes, Peroxidase*. New age International publishers, New Delhi, Pp: 100-101.
- Saha, R., Mandal, A. K. and Basu, R. N., 1990, Physiology of seed invigouration treatment in soybean (*Glycine max* L.). *Seed Science and Technology*, 18: 269-276.
- Sowmya, K. J., 2011, Studies on physiological, Biochemical and molecular aspects of seed invigouration in cucumber (*Cucumis sativus* L.). *Ph.D. Thesis* University of Agricultural sciences, Bangalore.
- Sung, F. J. M. and Chang, Y. H., 1993, Biochemical activities associated with priming of sweet corn seeds to improve vigour. *Seed Science and Technology*, 21: 97-105.

How to cite this article:

Manjunatha, B., B.C. Channakeshava, Y.G. Shadakshari, K. Bhanuprakash, K.R. Sreeramulu and Balakrishna, P. 2018. Influence of Seed Priming on Biochemical Changes in Fresh and Aged Seeds of Sunflower (*Helianthus annuus* L.) Hybrid KBSH-53. *Int.J.Curr.Microbiol.App.Sci*. 7(04): 3543-3551. doi: <https://doi.org/10.20546/ijcmas.2018.704.399>