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# BIOCHEMICAL CHANGES DURING ACCELERATED AGEING CONDITIONS OF MUNG BEAN SEEDS AND THEIR FIELD PERFORMANCE

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***Abstract***

*Mung bean seeds has been exposed to accelerated ageing(A.A) conditions( 45± 100% R.H.) for 2, 4, 8 and 14 days. Evaluation of biochemical changes were obtained by comparing accelerated aged seeds to un-aged seeds (control).The results were revealed many biochemical changes that have been occurred during accelerated ageing of seeds and leaves of field plants, such as: 1. A significant increase in malondialdehyde (MDA), as a result of membrane damage due to lipid peroxidation. 2. A significant increase in proline content of seeds exposed to A.A conditions compared to un-aged seeds. 3. A significant decrease in protein, catalase enzyme (CAT), glutathione (GSH) and ascorbate (AsA) in seed were exposed to A.A conditions. 4. No significant change of glutathione, ascorbate, proline, protein and chlorophyll content in leaves of filed plants of their seeds that already expposed to A.A conditions.*

***Key words:*** *aging, mungbean, catalase, glutathione, proline, ascorbate.*

## INTRODUCTION

Seed deterioration is a serious problem in developing countries where seeds are stored in places usually without a proper control of humidity and temperature. Temperature and seed moisture content (and/or relative humidity) are the main factors influencing seed deterioration and viability loss during storage (James, 1967). Changes occurring in seed during accelerated ageing are very significant with regard to quality and longevity of seed. Seed longevity is one of the components of seed quality (Milošević and Malešević, 2004; Baiyeri and Mbah, 2006). The speed at which ageing processes takes place depends on the seed's ability to resist degradation changes as well as its protection mechanisms (Balešević-Tubić, 2001). Many hypotheses have been proposed regarding causes of seed ageing such as lipid peroxidation mediated by free radicals, inactivation of enzymes or decrease in proteins, disintegration of cell membranes and genetic damage (McDonald, 1999; Murthy et al., 2003; Priestley, 1986).

Biochemical and physiological deterioration during seed ageing has been studied mostly under accelerated aging conditions using high temperature and high seed water content (McDonald, 1999; Hsu et al., 2003). Accelerated aging is one of the vigor tests widely used to determine the quality of seed lots (Thant et al., 2010). Under such storage conditions, seeds typically lose their viability within a few days or weeks. Although these studies allowed important progress towards the understanding of seed ageing mechanisms. The aim of the present research was to investigate the possible effects of accelerated ageing upon biochemical changes of mungbean seeds and its reflections on field plants performance.

## MATERIALS AND METHODS

### Determination of proteins

Seeds were extracted in 0.1M phosphate buffer (pH 5.6) and estimated by Bishop's method (Bishop et al, 1985). Bovine serum albumine (BSA) was used as standard protein.

### Determination of catalase activity

CAT was estimated by Aebi's method (Aebi, 1983). Seeds were extracted in 20 mM phosphate buffer (pH 7) then $H_2O_2$ added to the sample. Absorbance reading at 210 nm. Catalase activity determine by:

$$Catalase\ activity\ (unit) = \frac{reaction\ volume \times \Delta bs/min}{0.001}$$

**Determination of ascorbic acid**

Ascorbic acid was estimated in 2,4 dinitrophenyl hydrazine **(Shalata and Neumann, 2001)** One gram of seeds sample were ground with 10 ml of TCA solution and 2 ml of Metaphosphoric acid (m-$HPO_3$) was added and mixed well then 0.4 ml was added from DTCS reagent to the supernatant and incubated at 37°C for 3 hours. The tubes were cooled in ice for 10 minutes. Then 2 ml of cool $H_2SO_4$ (12 M) was added slowly to all tubes.Absorbance was read at 520 nm.

**Estimation of Malondialdehyde (MDA)Content**

MDA content was estimated by the method of Zacheo et al. (2000). One gram of seed sample was homogenized with 20 ml of Trichloroacetic acid. A volume of 1ml of 2-thiobarbituric acid (TBA) was added. Malondialdehyde was calculated according to the Beer-Lambert equation:

A= E*B*C

**Estimation of Glutathione Content (GSH)**

Glutathione was estimated by a modified method using Ellman's reagent **(**Ellman, 1959). A volume of 0.5 ml of seeds sample was ground with 5 ml of TCA 50%. A volume of 0.2 ml of TCA, 0.2 of a distal led water and 0.8 ml of Tris base buffer was added to test tube then 0.02 ml of DTNB reagent was added. The absorbance was read at 412 nm.

**RESULTS**

**1- MDA content**

The results of Figure 1 demonstrated that a significant increase of lipid peroxidation product (MDA) under accelerated aging treatment for (4, 8 and 14 day) compared to control. MDA content increased from 0.41µg\g (in control) to 0.53, 0.64 and 0.69 at 4, 8 and 14 day of aging respectively, although its increase at day 2 is not significant.

Accelerated ageing had significantly decreased the protein content in mungbean seeds, for 4,8,14 days compared to control (Figure 2-A), Whereas protein content was unchanged significantly in leaves of field plants (Figure 2-B) that already exposed their seeds to A.A conditions.

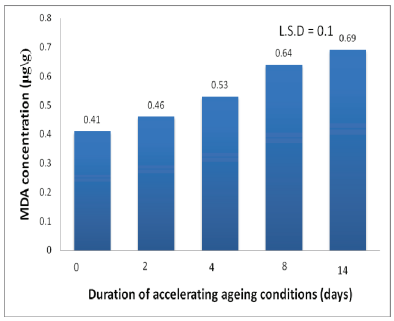
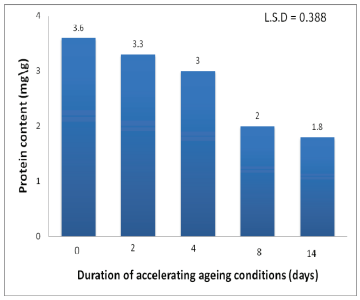
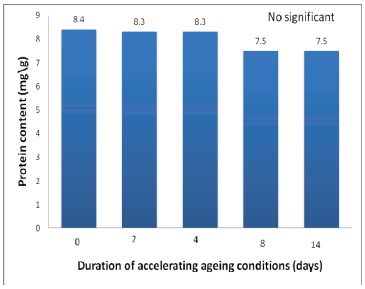



Figure 1. Effect of accelerated ageing conditions on MDA content (µg\g) of mung bean seeds.
2- protein content




A

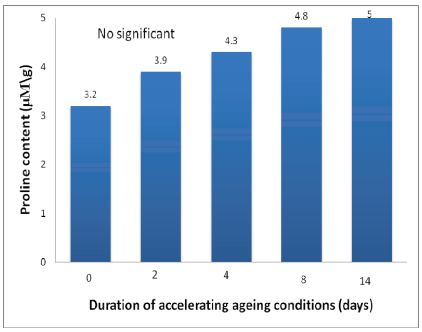


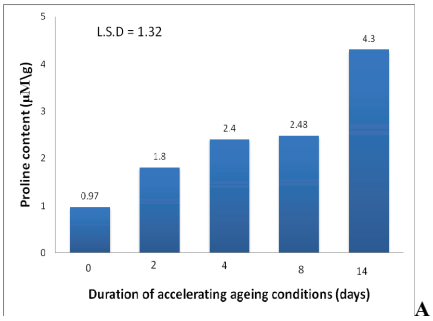

B

Figure 2. Effect of accelerated aging conditions on protein content (mg/g) of mung bean seed (2-A) and leaves (2-B), 3-proline content

Accelerating ageing conditions also exhibited significant effect on proline content compard to control.

Proline content increase with increasing accelerating ageing peroid as shows in figure 3-A. Whereas proline content was unchanged significantly in leaves of field plants (Figure 3-B), that already exposed their seeds to A.A conditions.




A

B

Figure 3. Effect of accelerated aging conditions on proline content (µM/g) of mung bean seed (3-A) and leaves (3-B)

## 2- Ascorbic acid

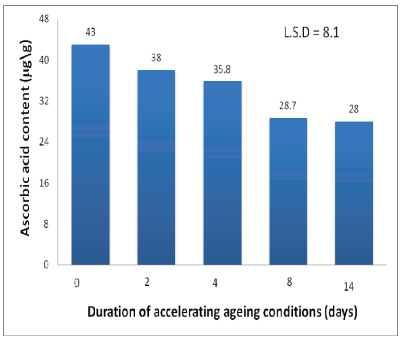
Figure (4-A) shows that accelerated ageing had significant decline in ascorbic acid of seeds after 8 and 14 days (28.7 and 28 µg/g) compared to control (43 µg/g). Whereas, ascorbate content was unchanged significantly in leaves of filed plants (Figure 4-B), that already exposed their seeds to A.A conditions.

## 3- Glutathione (GSH)

Accelerated ageing also decreased glutathione content of mungbean seeds. The significant reduction of GSH was started after 8 and 14 days of ageing treatment period (1543 and 1506 µg/g) repectively Figure 5.A. Whereas, GSH content was unchanged significantly in leaves of field plants (Figure 5.B) that already exposed their seeds to A.A conditions.

## 4- Catalase activity (CAT)

The reduction of CAT activity increased progressively with increasing ageing peroid. So, the least activity were recorded after 14 day (12.25 units) compared to control (73.7 units).

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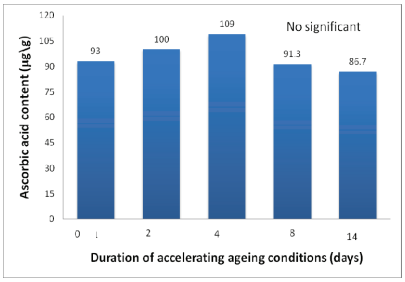


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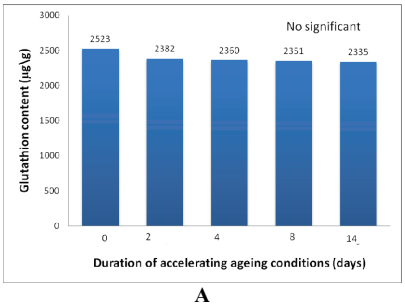
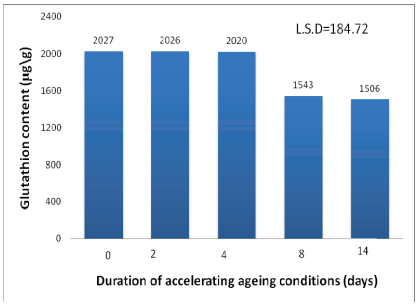
Figure 4. Effect of accelerated ageing conditions on ascorbic acid content (µg/g) of mung bean seed (4-A) and leaves (4-B)




A




B

Figure 5. Effect of accelerated ageing conditions on glutahtione conten (GSH) of mung bean seeds (5-A) and leaves (5-B)

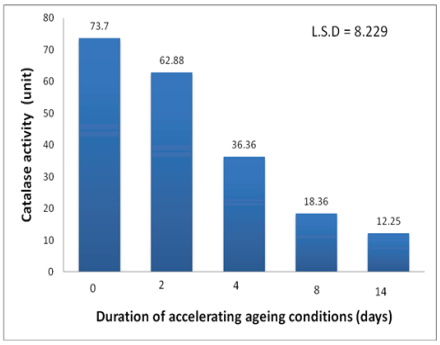



Figure 6. Effect of accelerated ageing conditions on catalase activity (unit) of mung bean seeds

**5- Chlorophyll content**

Figure 7 shows that accelerated ageing conditions of mung bean seeds has no significant changes on chlorophyll content of 5th true trifaliated leaves of field plants (after 56 day of sowing).

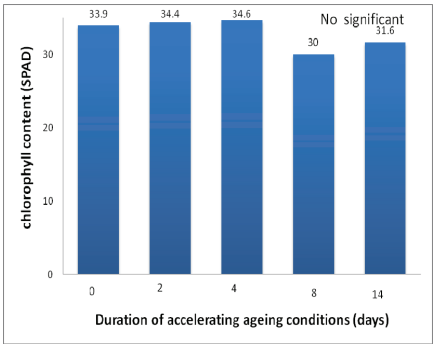



Figure 7. Effect of accelerated ageing conditions on chlorophyll content (spad)of mung bean leaves

**DISCUSSIONS**

The production of oxy-radicals or ROS by plant cells was induced by different factors such as: ageing, metabolic by-products, air-pollutants, herbisids, biotoxins and radiation (Scandalios, 1997). The latter was mentioned that, these radicals were causes damage for lipid and fatty acid, proteins, amino acids, pigments and nucleic acids. The above changes were reflects membrane damages and lossing of organelles functions in terms of permeability perturbation. Moreover, reducing the efficiency of cellular metabolism by reducing of carbon-fixation, as well as chromatid Mutations that collectively leads to the death of cell (Scandalios, 1997).

Four main points has been raised from the results of the current study.

First, accelerated ageing considered as oxidative stress (Scandalios, 1997; Blokhima et al., 2003) which induce the production of ROS that cause lipid peroxidation in terms of malon-dialdehyde (MDA) as a final product.

The results of the current study that deals with Mungbean seeds were confirmed this point (Figure 1) after 4 – days of A.A. conditions.

The decline in phospholipid content (or increase in MDA) content (Figure 1), is in agreement with increasing MDA content in many speices such as Soybean (Tubic et al; 2011), sunflower (Bailly et al.; 1998) and in sweet pepper (Kaewnaree et al., 2011).

The possible reason of this increment might be due to enhance lipid peroxidation products and subsequently resulted in membrane damage (Periestley et al., 1980). In addition, the increased seed leachates particularly in mung bean (Farah and Shaheed, 2012) confirms the above results, that associated with the loss of membrane phospholipid in deteriorated seeds (Copeland and McDonald, 1995), or due to increase of lipoxygenes activity during A.A conditions (Bhattacharjee et al., 2006; Shaheed et al., 2009). Similarly, a decrease in protein content was observed during A.A. conditions in pigenpea (Kelpana and Madhava Roa, 1997) and in rice (Kapoor et al., 2011).

The decrease was due to protein denaturation (Kaplana and Madhava Roa, 1995) and lack of ATP (Gidrol et al., 1998) or due to increase of protease activity (Bhattacharjee et al., 2006; Shaheed et al., 2009).

Second, the decline in enzymatic anti-oxydant defense system (e.g. Catalase activity, Figure 6) during the day-2- as well as non-enzymatic anti-oxydant defense system (e.g. Ascorbate, Figure 4) and Glutathione (Figure 5) during the day -4- and the day 8 respectively, denotes:

a) Precosious sensitivity of catalase during day -2- against high level of ROS as $H_2O_2$, which acts as substrate for the above enzyme (Fridovich, 1986). The loss of sunflower seed viability during accelerated ageing conditions was associated with a decrease in the activities of catalase enzyme (Hussein et al., 2012). However the decrease in CAT activity appered to be associated with an increase in the level of MDA (Figure 1). Similar results were reported

in sunflower seeds (Balesevic et al., 2005) and maize seeds (Wattanakal Pakin et al., 2012). In addition, A.A. may either denaturates the enzymes to different degrees or affects their synthesis (Bailly et al., 1998).

b) The need for Ascorbate as non-enzematic anti-oxidant during the day -4- of A.A. conditions, that coincided with increased level of MDA (Figure 1) and reflects the damage of phospholipid by lipid peroidation, as well as the decline in protein (Figure 2).

c) The delayed need (requirement) for GSH until the day -8- of A.A conditions (Figure 5). It was confirming the progression of events after exposing seeds to A.A conditions that enhances the anti-oxidant defense mechanisms to acts according to its priority for each event. Accelerated ageing conditions for 14 day caused a sever diminishing of AsA&GsH contnt (92.5% and 97.5% respectively) in maize seeds (Hussein et al., 2012).

d) The latter found a positive correlation (r = 0.9966) between AsA & GSH in maize as well as (r = 0.9899) in sunflower seeds. Similar results were reported in sunflower seeds (De Paula et al; 1996) and in senescent leaves of pea (Jimenez et al; 1998). This reduction was probably due to the decrease in Glutathione reductane (GR) activity (Hussein et al., 2012) that took place under the A.A. conditions (De Puala etal, 1996; Jimenez et al., 1998) or due to the increase in ascorbic acid oxidase (Hussein et al., 2012).

Third, Proline level was raised at day -4- (Figure 3), that coincided with the diminishing of phospholipid (Figure 1) and protein (Figure 2) (Ain-Lhout et al., 2001).

Although, proline has many physiological roles in plants but, it was assumed that proline ats as electron acceptor to avoid the damage of photosynthesis (in terms of unchange chlorophyll content Figure 7) that inhibited by ROS (Hare et al., 1999). Alternatively, proline maintaining high level of GSH as well as the enzyme that deals with GSH metabolism (Hussein et al., 2012).

Fourth, the unchanged level of protein (Figure 2-b), Ascorbate (Figure 4-b), proline (Figure 3-b) and chlorophyll (Figure 7) in leaves (5 $^{th}$ true trifolated leaf) of field plants of mung bean suggested the strength (Seed Vigor) of some seeds within a seed lots having a high performance after 56- days, irrespective of A.A. conditions.

The standard germination test may fail to provide accurate information concerning a seed lots field performance potential for at least few reasons. Mostly, a test was designed to provide for a first and final count. The first count deals with strong seeds that already germinated, the final count is designed to provide a sufficiently long period that every opportunity to be considered terminable, particularly when provided environmental stresses associated with field emergence (AOSA, 2000).

## CONCLUSIONS

It could be concluded that accelerated ageing caused a significant increase in MDA and proline, while it caused a decreases in protein CAT, GSH and AsA.

## REFERENCES


Abass F.A., 2013. Effect of accelerated ageing conditions of Mung bean seeds on growth and production. MSC. thesis, Univ. of Babylon, Iraq.

Aebi H., 1983. Catalase in vitro, Methods Enzymo., 105:121-126.

Ani-Lhout F., Zunzunegui F.A, Diaz Barradas M.C, Tirado R., Clavijio A., Garcia Novo F., 2001. Comparison of proline accumulation in two Mediterranean shrubs subjected to natural& experimental water deficit". Plant Soi.,l 230:175-83.

Association of Official Seed Analysts (AOSA), 2000. Rules for testing seeds. J. Seed Tech., 16:16-17.

Bailly C., Abdeliah B., Francoise C., Daniel C., 1998. Free radical scavenging as affected by accelerated ageing & subsequent priming in sunflower seeds. Physiol. Plant., 104:646-652.

Baiyeri K.P., Mbah B.N., 2006. Surface sterilization and duration of seed storage influenced emergence and seedling quality of African breadfruit (*Treculia africana* Decne). Afr. J. Biotech., 5(15):1393-1396.

Balešević-Tubić S., 2001. The influence of ageing processes on seed viability and biochemical changes in sunflower seed. PhD Thesis – University of Novi Sad, Novi Sad.

Balešević-Tubić S., Malenčić Đ., Tatić M., Miladinović J., 2005. Influence of ageing processes on biochemical changes in sunflower seed. Helia., 28(42):107-114.

Bates L.S., Waldes R.P., Teare T.D., 1973. Rapid determination of Free Proline for water stress studies. Plant & Soil, 39:205-207.

Bhattacharjee A., Kanp U.K., Chakabarti D., Pati C.K., 2006. Technique for storage longevity of Mung bean & Sunflower seeds using sodium dikegulac & Eucalyptus oil. Bangladesh J. Bot., 35(1):55-61.

Bishop M.C., Dben-Von Laufer J.L., Fody E.P., 1985. Thirty three Contributors. Clinical Chemistry Principles, Procedures & Correlations, p. 181-182.

Blokhina O., Virolainen E., Fagerstedt K.V., 2003. Antioxidants, oxidative damage & oxygen deprivation stress. Rev .Ann. Bot., 91:179-194.

Clavijio A,, Garcia Novo F., 2001. Comparison of proline accumulation in two Mediterranean shrubs subjected to natural & experimental water deficit. Plant Soil 230:175-183.

Copeland L.O., McDonald M.B., 1995. Principles of Seed Science and Technology. Chapaman and Hall, p. 115. Fifth Avenue, New York, USA.

DePaula M., Perez-Otaola M., Darder M., Torres M., Frutos G., Martinez-Honduvilla C.J., 1996. Function of the ascorbate-glutathione cycle in aged sunflower seeds. Physiol. Plant., 96:543-550.

Ellman G.L., 1959. Arch. Biochem. Biophys., 822,70.In: Shalata A., Neumann P.M., 2001. Exogenous ascorbic acid (vitamin C) increases resistance to salt stress & reduces lipid peroxidation. J. Exp. Bot., 52: 2207-2211.

Fridovich I., 1986. Superoxide dismutases.Adv Enzymol.Relat. Areas Mol. Biol. 58:61-97.

Gidrol X., Serghini H., Noubhani A., Mocoquot B,. Mazliak P., 1989. Biochemical changes induced by accelerated ageing in sunflower seeds. I. Lipid peroxidation & membrane damage. Physiol. Plant, 76: 591-597.

Hare P.D., Cress W.A., Van Staden J., 1999. Proline synthesis & degradation: a model system for elucidating stress-related signal transduction. J. Exp. Bot., 50:413-434.

Hsu C.C., Chen C.L., Chen J.J., Sung J.M., 2003. Accelerated ageing-enhanced lipid peroxidation in bitter gourd seeds and effects of priming and hot water soaking treatments. Sci. Horticult. 98:201-212

Hussien J.H., 2012. Physiological and Biochemical Changes in Maize and Sunflower Seeds during Accelerated Ageing Conditions. Ph.D.thesis, Univ. of Babylon, Iraq.

James E., 1967. Preservation and seed stocks. Adv. Agron., 19:87-106.

Kaewnaree P., Vichitphan S., Klanrit P., Siri B., Vichphan K., 2011. Effect of accelerated ageing process on seed quality & biochemical changes in sweet pepper (*Capsicum annuum* Linn.) seeds. Biotech., 10(2):175-182.

Kalpana R., Madhava Rao K.V., 1995. On the ageing mechanism in pigeonpea (*Cajanus cajan* L. Millsp.) seeds. Seed Sci. and Technol., 23:1-9.

Kalpana R., Madhava Rao K.V., 1997. Protein metabolism of seeds of pigeonpea (*Cajanus cajan* L. Millsp.) cultivars during accelerated ageing. Seed Sci. and Tech., 25:271-279.

Kapoor N., Arya A., Siddiqui Mohd. Asif, Kumar H., Amir A., 2011. Physiological & Biochemical Changes During Seed Deterioration in Aged Seeds of Rice (*Oryza sativa* L.). Amer. J. of Plant Physiol., 6(1):28-35.

McDonald M.B., 1999. Seed deterioration: physiology, repair and assessment. Seed Sci. Tech., 27:177-237.

Milošević M., Malešević M., 2004. Seed production. Institute of Field and Vegetable Crops, Novi Sad., p. 110-114.

Murthy U.M., Kumar N.P.P., Sun W.Q., 2003. Mechanisms of seed ageing under different storage conditions for *Vigna radiata* (L.) Wilczek: Lipid peroxidation, sugar hydrolysis, Maillard reactions & their relationship to glass state transition. J. Exp. Bot., 54:1057-1067.

Priestley D.A., 1986. Seed Ageing: Implications for Seed Storage & Persistence in the Soil. Cornell University Press, Ithaca, New York.

Priestley D.A., McBridge M.B.. Leopold A.C., 1980. Tocopherol, organic free radical levels in soybean seeds during natural and accelerated ageing. Plant Physiol., 66:715-719.

Scandalios J.G., 1997. Oxidative Stress and the Molecular Biology of Antioxidant Defenses. Cold Spring Harbor Laboratory Press, New York, p. 480.

Shaheed A.I., Jabor M.A., 2009. Effect of ageing on permeability perturbation in Mung bean (*Phaseolus aureus* Roxb.) cuttings and its control by supplying parsley extract. I: Membrane structure and efflux Int. J. Chem. Sci . 7(3):2071-2086.

Shalata A., Neumann P.M., 2001. Exogenous ascorbic acid (vitamin C) increases resistance to salt stress and reduces lipid peroxidation. J. Exp. Bot., 52:2207-2211.

Thant K.H., Duangpatra J., Romkaew K., 2010. Appropriate Temperature and Time for an Accelerated Aging Vigor Test in Sesame (*Sesamum indicum* L.) Seed. J. Nat. Sci., 44:10-16.

Tubic S.D., Tatic M., Dordevic V., Nikolic Z., Subic J., Dukic V., 2011. Changes in soybean seeds as affected by accelerated and natural ageing. Romanian Biotechnological Letters, 16(6):6740-6747.

Wattanakulpakin P., Photchanachai S., Ratanakhanokchai K., Lay Kyu K., Ritthichai P., Miyagawa S., 2012. Hydro-priming effects on carbohydrate metabolism, antioxidant enzyme activity and seed vigor of maize (*Zea mays* L.). African J. Biotech., 11(15):3537-3547.

Zacheo G., Cappello M.S., Gallo A., Santino A., Cappelo A.R., 2000. Changes associated with post-harvest ageing in Almond seeds. Lebensm-Wiss U. Technol., 33:415-423.