IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Utilization of Cotyledonary Contents and the Respective Enzyme Activities in it: A Case Study of *Trigonella foenum-graecum* L.

Sweta Gaikwad*, Kavita Sharma

Department of Botany, Govt. Arts and Commerce Girls' College,

Devendra Nagar, Raipur.-492009, India.

Study Area: Raipur, Chhattisgarh, India

Coordinates: 21.25^0 N; 81.63^0E

Abstract:

Fenugreek is one of the well-known species in human use. Its seeds and green leaves are used as food and fodder as well as in medicinal preparation which is an old practice of human history. The seeds are traditionally used for the treatment of many diseases. Fenugreek seeds are also used as spice and condiment. Besides this it is also nutritionally very rich. In the present study, the extraction and estimation of the important reserve nutrients and three major hydrolases, Amylase, Protease and Lipase were done at all the six important intermediate phases of germination. The percentage of hydrolases were found to vary at different phases of germination. More starch was degraded during early phases of germination. Initial phases showed rapid protein degradation. Protein mobilization into the axis lowered its level in cotyledons due to *in situ* utilization of soluble protein and reduction of nitrates into plant proteins. Lipase activity was higher during initial phases due to more availability of water for stored lipid hydrolysis. These facts would be helpful in the encouragement of fenugreek consumption as a nutritional supplement along with its medicinal properties. It will also be useful in detailed study of utilization of cotyledonary contents of fenugreek and other similar seeds trough out the phenomenon of germination.

Key words: Legume seeds, Amylase, Protease, Lipase, Cotyledons.

Introduction:

Seeds can be considered as the most important part of a seeded plant and its life cycle.The plant seed is not only an organ of propagation and dispersal but also the major plant tissue harvested by mankind (Ramakrishna & Rao, 2004). A seed germination is an important process in the life cycle of plants and it is initiated when the apparent metabolic dormancy of desiccated seeds is disrupted by imbibitions. It leads to an extensive break down of stored carbohydrates, lipids and proteins in the storage organs of seeds to provide energy and other nutritional requirements of the growing embryo (Botcha *et al.*, 2011). In leguminous seeds, food is stored mainly in the cotyledons and there is no endosperm. Thus, endosperm gets completely utilized by the growing or developing embryo.

In leguminous seeds, carbohydrates, proteins and fats are stored mainly in the cotyledons (Gorecki *et al*., 2000). The amount of these reserves make some seeds nutritionally much more rich in comparison to others. Reserve food is broken down by the formation of various types of hydrolases like, amylases, proteases, nucleases, lipases, etc. However the food inside the seed is not in the form of simple sugars, instead of in the form of complex, insoluble molecules such as protein and starch. Because of this, mobilization takes place to convert the molecules in to something useful to the plant. Mobilization happens because of enzymes which digest the
large molecules. So enzymes are large biological molecules responsible for the thousands of chemical interconversions that sustain life. Just like any other leguminous seeds obviously, the seeds of *Trigonella foenum-graecum* L. also utilize the stored food materials at various stage of seedling growth and development. In the present study, an attempt to analyze the nutritional value and the rate of three major hydrolases with respect to all the important intermediate phases of germination has been taken.

Methodology:

Trigonella foenum-graecum L. is an erect annual leguminous herb originally from South-Eastern Europe and Western Asia, but grown now mainly in India and also in certain parts of Asia, Northern Africa, Europe and United States Altuntas *et al*., (2005). Its seeds and green leaves are used as food and fodder as well as in medicinal preparation which is an old practice of human history. It provides natural food fibre and other nutrients required in human body Thomas *et al.,* (2011). The seeds are traditionally used for the treatment of many diseases. Fenugreek seeds are also used as spice and condiment. India is a major producer of fenugreek and also a major consumer of it for its culinary uses and medicinal application. It is used in physiological utilization such as antibacterial, anticancer, antiulcer, hypocholesterolemic, hypoglycemic, antioxidant and antidiabetic agent.

As the first step of our study, the nutritional value of fenugreek was determined by extracting and estimating the major cotyledonary reserves (carbohydrates, proteins and lipids) of the seeds after 24hr of imbibition followed by extraction and estimation of the three major hydrolases (amylase, protease and lipase) at all the important intermediate phases of epigeal germination through the following described steps.

Healthy, good quality and high yielding variety of fenugreek seeds were purchased from the local market of Bhilai, Chhattishgarh, India. The selected healthy seeds of uniform size were germinated following Misra & Kar (2002). The weight of each pair of cotyledons was found to be16 mg.

Six most important phases of germination were considered as :

"I"- Phase of Imbibition (attained after 24hr of imbibition in normal tap water).

"R"- Phase of Radicle expansion (attained after 48hr of germination).

"H"-Phase of Hypocotyl extension (attained after 72hr of germination).

"B"- Phase of Branching in radicle (attained after 96hr of germination).

"E"- Phase of Epicotyl extension (attained after 120hr of germination).

"L"- Phase of unfolding of first Leaves (attained after 144hr of germination).

Nutritional value of the fenugreek seeds was evaluated by estimating carbohydrates, as total sugar and starch, protein and lipid content in 24hr of imbibed seeds. Healthy, uniform and high yielding seeds were imbibed in water in petriplates. After 24hr, the imbibed seeds were taken and their cotyledons were removed carefully and biochemical estimations were done in the cotyledons. All the biochemical estimations were done in four replicates and the average value was calculated in the total weight of per pair of cotyledons in *Trigonella foenum-graecum* L.

Sugar in the imbibed seeds was estimated by Anthrone method following Plummer (2011). Estimation of Starch was done by using Anthrone method by following Plummer (2011).
Protein content of imbibed seeds of *Trigonella foenum-graecum* L. was estimated by using Folin-Lowry method following Plummer (2011). Lipid content of the seeds was evaluated by using Becker's method (Becker *et al*., 1978, Botcha *et al.*, 2011).

In the next step, the seeds were analyzed for the enzymatic activities during the process of germination, considering Amylases, Proteases and Lipases separately. The biochemical estimations were done with four replicates and the average values were calculated with respect to the total weight of a pair of cotyledons of the fenugreek seeds.
Estimation of Amylase activity was done by using DNS-reagent method by following Plummer (2011) as followed by Oboh (2005).
Protease activity in the germinating cotyledons of *Trigonella foenum-graecum*L. was estimated by using azocasein as a standard by following the method of Nigam and Ayyagari, (2007) as followed by Arunachalam and Saritha (2009).
Lipase activity was estimated by using the method suggested by Nigam and Ayyagari (2007) as also followed by Nahak et al.(2010).

Results:

All the above extractions and estimations were done at all the six intermediate phases taken under consideration separately. Analysis of nutritional value of the seeds revealed the following results. i) They contained 0.17mg. of sugar / pair of cotyledons (1.1%). ii) 0.91mg. of total starch / pair of cotyledons (5.7%). iii) 4.16mg. of total protein /pair of cotyledons (26.0%) and iv) 1.13mg. of total lipid / pair of cotyledons (7.06%).

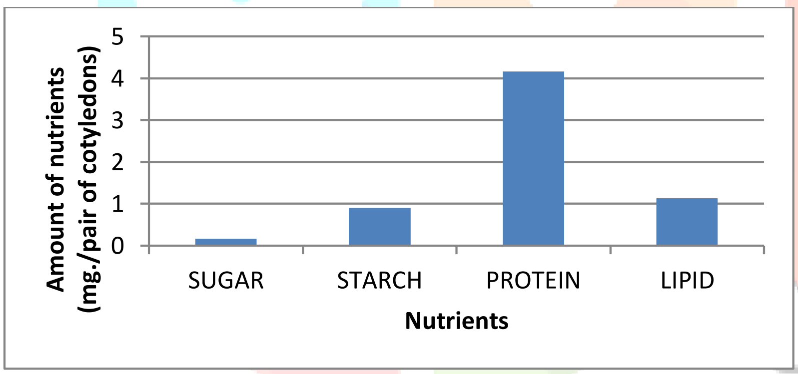

Figure-1: Nutritional value of *Trigonella foenum-graecum* L. seeds inmg./pair of cotyledons at "I" phase of germination.

During the experiment, the values for Amylase activity (units/pair of cotyledons) of *Trigonella foenum-graecum* L.seeds ranged from 8.9 to 10.4 (from the first phase "I" to the last phase "L") during the process of germination. The Amylase activity increased upto phase "B" (Phase of Epicotyl extension) in germinating cotyledons. The maximum value and minimum value were recorded at phase "B" and "I" respectively. ANOVA result revealed significant differences among all the phases of activities. Except the phases "H" and "L" rest all the phases were statistically differed from each other.

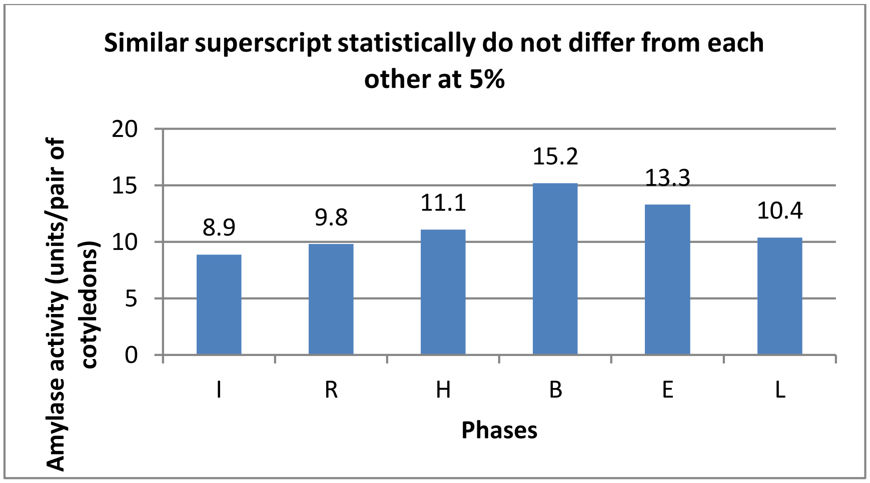

Figure-2: Amylase activities of *Trigonella foenum-graecum* L. seeds during the different phases of germination.

An increase of protease activity was noticed when the seed went through the initial phase of germination that is change from phase “I” to “B”. Suddenly the rate of protease activity decreased during the phase “B” to “L”. This decrease in the rate of activity was calculated 23% and thus phase “B” was considered to be the peak point of the enzyme action after which the rate was decreased. ANOVA result revealed significant differences among all the phases of activities.

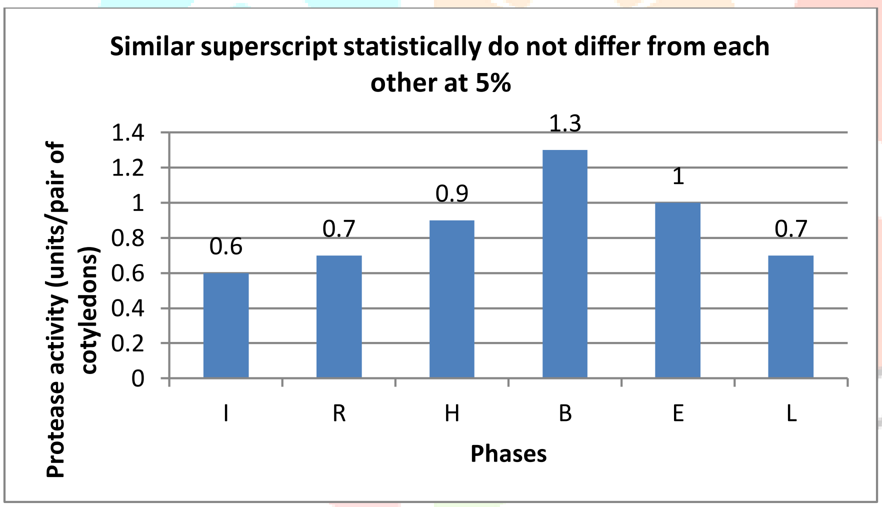

Figure-3:Protease activity of *Trigonella foenum-graecum* L. seeds during different phases of germination.

Increase in Lipase activity was highest during the phase “H” and then rate of enzyme action slowed down. In the initial stages of germination, the enzyme Lipase acted rapidly. Increase of about 80% in the rate of enzyme action was recorded from phase change “I” to “R”. ANOVA result revealed significant differences among all the phases of activities. All the phases were statistically differred from each other.

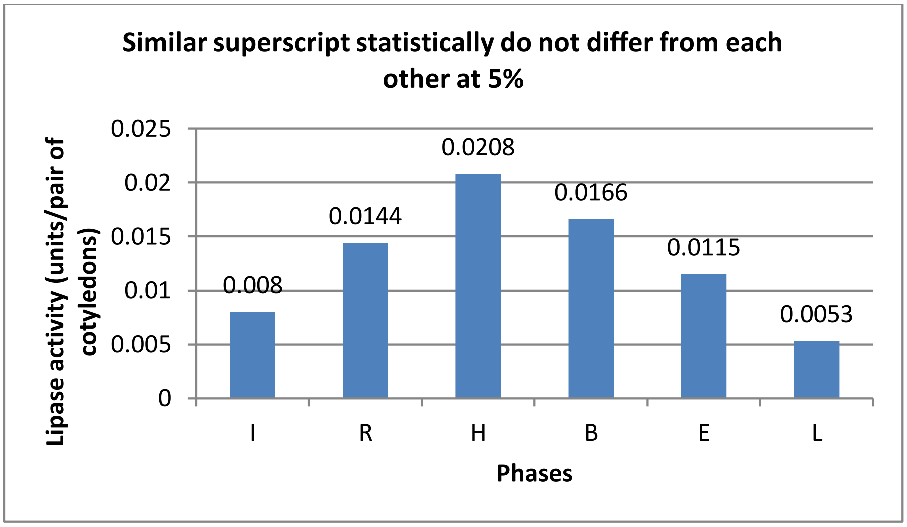

Figure-4:Lipase activity of *Trigonella foenum-graecum* L. seeds during different phases of germination.

Discussion:

Nutritional value of any seed is determined by analyzing and estimating all the vital nutrients including total carbohydrates, proteins, fats or lipids, dietary fibres whereas, vitamins and minerals are also taken into consideration by Finney (1982) in determination of the nutritional value of different seeds. In this investigation, nutritional value was determined in the fully imbibed seeds, because after 24hrs of imbibition, it was easy to separate the seed coats from *Trigonella foenum-graecum* L. and the nutrients present in the cotyledons of the seed could be determined easily.

The nutritional value of imbibed *Trigonella foenum-graecum* L.cotyledons, indicated maximum protein quantity 4.16mg./pair of cotyledons that was 26% of total cotyledonary weight. This concluded that the seeds are very proteinaceous and can be used as a good source of edible protein in daily diet. Similar estimation of total protein was made by Meghwal and Goswamy (2012), in *Trigonella foenum-graecum* L. Total carbohydrate content of *Trigonella foenum-graecum* L. seeds was estimated to be 42.3gm/100gm was similar to the result obtained in present investigation. The results obtained for protein content of imbibed seeds showed an interesting fact that was somehow different from the results obtained for total protein content of germinated and ungerminated seeds of *Trigonella foenum-graecum* L. as conduted by Shakuntala *et al*. (2011), which were 39.28% and 48.2% respectively. Whereas, the respective result of present study was 26%. It was because of the fact that imbibition and then the physiological or biochemical changes related to germination decreases the level of protein inside the cotyledons due to their mobilization and successive utilization during the process.

Starch degradation and its metabolism were studied extensively in many kinds of cereal and some legumes by Bewley & Black (1994). During germination of several species, amylase activity increases in the cotyledons and the starch content decreases (Prakash *et al.*, 2015). There are two mechanisms for starch degradation; i) amylitic- involving amylases, ii) the phosphorolytic- involving phosphorylase. The amylitic pathway is the major pathway for degradation of cotyledonary starch reserves in pea and similar legumes (Murtaza & Asghar, 2012).

In case of *Trigonella foenum-graecum* L., the amylase activity increased upto the phase "B" (96hr of germination). The maximum increase in sugar level was observed between the phase "H" and "B" and this rise in sugar level was about 53%. After this period, activity again declined rapidly towards the next phase. The loss of activity was 48% from "B" to "E". It might be signifying that the amount of starch present per pair of cotyledons in the seeds was much more. It was known that amylase activity was directly proportional to the starch content. So this condition encompassed within its span of rapid starch breakdown indicated its importance in the mobilization of starch reserves in the cotyledons. Amylase activity was found to be lower in cotyledons. Thus it represented that the amylase was the major pathway of starch degradation in the axis and not that much significant in cotyledons of germinating seeds. Bhushan & Gupta (2008) reported similar results in oat seeds and demonstrated that during germination, amylase in the cotyledons increased rapidly from 4^{th} to 10^{th} day and then level off.

Increase in activity of proteases was correlated with the breakdown of storage proteins supported that these proteases were responsible for protein degradation Ramakrishna & Rao (2004). Mobilization of storage protein in germinating seeds was initiated by endoproteases which converted the water-insoluble storage protein into soluble peptides that could be further hydrolyzed to amino acids by exopeptidases (Ramakrishna & Rao, 2005). Protease activity of brown rice increased seven folds during 7 days of germination and showed the highest rate on day 6 when determined at p^H 3.5 by Li *et al*. (2011), while in germinating *Trigonella foenum-graecum* L. cotyledons, the rate of protease activity increased up to the phase "B" , that happened after 96hr (4 days) of germination. After this phase, the rate of proteolytic activity started decreasing towards the successive phases, "E" and "L". Maximum increase in rate of protein degradation occurred from phase "H" to "B" i.e. between 72hr to 96hr of germination and this gain in activity was recorded 44.4%. Before this, increase of 16.6% and 28.5% was observed between phases "I" and "R" and then from "R" to "H" respectively. The difference in *Trigonella foenumgraecum* L. was might be due to its smaller size as well as relatively faster rate of germination that the reserve proteins got themselves degraded comparatively faster. Softer or thinner seed coat was may be another reason behind this. After certain time period the rate of degradation became slower and enzyme activity falls down. Investigations made by Akhtaruzzaman *et al*. (2012) on protease activity in seven leguminous seeds, soybean, lentil, black gram, green gram, Bengal gram, groundnut and pea bean revealed almost the similar results.

Lipase enzyme breaks down or degrades the complex insoluble lipids into simpler fatty acids and alcohol which can be easily carried to the point of axis growth and become utilized by it. Some seeds germinate faster than others due to more lipid accumulation and further mobilization of it. These seeds germinate faster due to higher lipase activity in degrading the stored lipid in the cotyledons, while others give a poor response (Cantisen *et al*., 1999). Possibly it is due to the slower utilization of reserve lipids by the embryo for its growth during seed germination. The proper utilization of lipid by the embryo is solely dependent on one of the major hydrolases responsible for its degradation and that is lipase. In our study, the lipid change and the mode of lipase activity revealed similar results as obtained by Munshi *et al*. (2007) and Nahak *et al*. (2010). A very high rate of lipid degradation was observed at the initial phases of germination in the seeds. There was a gain of about 80% in the lipase activity between the phases "I" and "R". This rate of increase in activity was recorded as 44.4% for phase "R" to "H" in germinating cotyledons of *Trigonella foenum-graecum* L. This increase in activity continued up to the phase "H" which was attained after 72hr of germination. Then a decrease of 20.2%, 30.7% and 54% was recorded for change of phase "H" to "B", "B" to "E" and "E" to "L" respectively. Maximum rate of activity was observed from "I" to "R". This was because of the insoluble lipids, especially fats started breaking down after imbibition which resulted in the swelling of the seed, breaking of the seed coats and resulting radicle expansion or emergence. This entire phenomenon required most of the reserved nutritional energy and in order to satisfy the need of the growing seedling.

The rate of germination was directly proportional to the liquid content and its mobilization and this relation were seen earlier in the seedlings of sunflower (Bahri, 2000). A higher amount of lipid was observed in the embryonic axes of fast-growing seeds of sunflower on the 8^{th} and 10^{th} day of germination. It was due to enhanced fatty acid synthesis and due to lipase activity. Nguyen *et al*. (2006) emphasized that germinating seeds synthesized fatty acids from lipids (fats) by the enzyme lipase which was used in the formation of membrane lipids in the growing embryos. Thus an increase in lipid content was seen in the axis of seedlings during germination observed by Munshi *et al*. (2007). Similar observations were made by Gadge *et al*. (2011) in oil seeds of *Glycine max*. While in seven other seeds from seven different species namely, castor bean, peanut, sunflower, cucumber, cotton, corn and tomato, the storage tissues of all these seeds except castor bean contained only alkaline lipase activity which increased drastically during germination, confirmed Sana *et al*. (2004).

Analysis of major food reserves of the fenugreek seeds revealed it as a nutritionally rich one rather its worldwide medicinal importance. Study on hydrolases and their activities expressed the role of such enzymes in the utilization of cotyledonary content in germination and seedling development in the seeds. Amylosis was the major pathway of starch hydrolysis. Very little amylase activity persisted in the early stages of germination. So most of the amylase was synthesized de novo in the cotyledons. Mobilization of storage protein in leguminous seeds represented one of the most important post-germinative events in the growth and development of seedling. Proteolytic enzymes, mostly proteases played the central role in mobilization and utilization of reserve protein. Complex protein hydrolysis into simpler and soluble peptides was faster in the early stages of germination and became slower afterwards in later phases. Maximum activity occurred between the phases of hypocotyl extension ("H") and branching in radicle ("B") as this was physiologically the most active period of germination. Utilization of stored lipids especially fats in the leguminous seeds was monitored by estimation of lipase activity. The enzyme got activated as soon as water became available to the inner contents of the seed by imbibition. These facts regarding change in the hydrolytic enzymes during seed germination would be helpful in developing new technologies to improve germination percentage of many seeds.

Acknowledgements:
First author is thankful to the Principal, Govt. Nagarjuna P.G. College of Science, Raipur, C.G. for his support and positive inspiration. Thanks, are also due for the Principal, Ravenshaw College, Cuttack, Odisha for his kind support as a portion of this study was conducted there.

References:

Akhtaruzzaman, M.,Mozumder, N.H.M.R.,Jamal,R., Rahman, A. and Rahman,T. (2012): Isolation and characterization of protease enzyme from leguminous seeds. *Agricultural Science Research Journals*, 2(8):434-440.

Altuntas,E., Engin Ozgoz, O. and Taser,F.(2005): Some physical properties of fenugreek seeds. *Jour. of Food Engineering*,71:37-43.

Arunachalam,C. and Saritha,K. (2009): Protease enzyme: an eco-friendly alternative for leather industry. *Indian Journal of Science and Technology*. Vol.2, No. 12:29-32.

Bahri,S.(2000): Lipase activity in germinating sunflower seedlings. *Biochem. Soc.Trans*. 28(6):771-773.

Becker,W.M., Leaver, C.J. Weir, E.M. and Riezman, H.(1978): Regulation of glyoxysomes enzyme during germination of cucumber. Dev. Changes in Coty. Prot.,RNA and enz. activity during germination. *Plant Physiology*,62:542-549.

Bewley, J.D. and Black, M.(1994): Seeds: Physiology of development and germination. Pub. by: *Plenum Press, New York*,USA.445p.

Bhushan, B. & Gupta, K. (2008): Effect of lead on carbohydrate mobilization in oat seeds during germination. *J. Appl. Sci.Environ. Manage*., 12 (2):29-33.

Botcha, S., Prattipati, S. and Atluru, A.(2011): Biochemical changes during seed germination of *Sterculia urens* Roxb. *Not. Sci. Biol.*, 3(3):105-108.

Cantisen, S., Force, E.M., Ortega, R.A. and Garces, R.(1999): Lipid Characterization in vegetable tissues of high-saturated fatty acid sunflower mutants. *Journal of Agriculture and Food Chemistry,* 47:78-82.

Gadge, P.P., Madhikar, S.D., Yewle, J.N., Jadhav, U.U., Chougale, A.D., Zambare, V.P. & Padul, M.V. (2011): Biochemical Studies of lipase from germinating oil seeds (*Glycine max*). Am. J. of Biochem. Biotechnol., 7(3):141-145.

Gorecki, R.J., Fordonski, G., Halmajan, H., Horbowicz, M., Jones, R.G. and Lahuta, L.B.(2000): Seed physiology and biochemistry, 138-143 pp. In, Hedley, C.L.(eds). Carbohydrates in grain legume seeds, seed Physiology and Biochemistry. CABebook, Oxon.

Li, C., Cao, X., Gu, Z. and Wen, H.(2011): A preliminary study of the protease activities in germinating brown rice (*Oryza sativa* L.). *Journal of the Science of Food and Agriculture*, 91(5):915-920.

Meghwal, M. and Goswamy,T.K.(2012): A review on the functional properties, nutritional content, medicinal utilization and potential application of fenugreek. *J. Food Processing and Tech.*, 3(9):181-190.

Misra, N.M. and Kar, R.K.(2002): Effect of light, GA3 and kinetin on amylase formation in cotyledons of germinating cowpea. *Geobios*, 29:125-128.

Munshi, S.K., Sandhu, S. and Sharma, S.(2007): Lipid composition in fast and slow germinating sunflower (*Helianthus annus* L.) seeds. *Journal of Gen. Appl Plant Physiol.*, 33(3-4):235-246.

Murtaza, G. & Asghar, R. (2012): α- Amylase activities during seed development and germination in pea treated with salicylic acid. *Pak. J. Bot*., 44(6):1823-1829.

Nahak, G., Mohanty, C.K., Mohapatra, N.K. and Sahu,R.K.(2010): Enzymatic transesterification of lipases obtained from bacterial and plant sources. *Conti. J. Biomed. Sciences*, 4:21-36.

Nigam, A. and Ayyagari, A.(2007): ***Lab Mannual of Biochemistry,Immunology and Biotechnology*****.** Pub. by: Tata McGraw Hill, New Delhi.358p.

Nguyen, Q.T., Kisiala, A. & Narine, S. (2016): Seed development: Fatty acids and phytohormone metabolism and their interactions. *Curr. Genomics*. 17(3):241-260.

Oboh, G. (2005): Isolation and characterization of amylase from fermented cassava (*Manihot esculenta* Crantz.) wastewater. *African J. Biotech.*, 4(10):1117-1123.

Prakash, S., Sharma, S. Yadav, K. & Yadav, R. (2015): Biochemical Analysis of Amylases During Germination of Buckwheat (*Fagopyrum esculentum*) Seeds: A Pharmaceutical Plant. *Int. J.Sci. Res. Methodol.*,2(1):31-46.

Plummer, D.T.(2011): ***An Introduction to Practical Biochemistry***. Tata McGraw Hill Edition, New Delhi.318p.

Ramakrishna, V. and Rao, R. P.(2004): Storage protein degradation in germinating Indian bean (Dolichos lablab L. var.lignosus) seeds. *Seed Sci. Technol*.,34(1):161-168.

Ramakrishna, V. and Rao, R.P.(2005): Purification of acidic protease from the cotyledons of germinating Indian bean (*Dolichos lablab* L. var. lignosus) seeds. *Af. J. Biotech.*, 4(7):703-707.

Sana, N.K., Hossin, I., Haque, E.M. and Shaha, R.K. (2004): Identification, Purofication and Characterization of Lipase From Germinating Oil Seeds (*Brassica napus* L.). *Pak. J. Biol. Sci*., 7(2):246-252.

Shakuntala, S., Pura Naik, J.,Jeyrani, T., Naidu, M.M. and Srinivas, P.(2011): Characterization of germinating fenugreek (*Trigonella foenum-graecum* L.) seed fractions. *Int. J. Food Sci. and Tech.*,46:2337-2343.

Thomas, J.E., Banclara, M.,Lee, E.L., Driedger, D. and Acharya, S.(2011): Biochemically monitoring fenugreek to develop functional food and medicinal plant variants. *N. Biotech.*, 28:110-117.

Finney, P.L.(1982): Effect of germination on cereal and legume nutrient changes and food or feed value: a comprehensive review. *Recent Advances In Phytochemistry,Plenum Press, New York*, 17:229-305.