

BIOCHEMICAL CHANGES OCCURS IN THE SEEDS OF *PONGAMIA PINNATA* (L.) PIERRE BY FUNGAL INFECTION

Dwarka D. J. and Rajasab A.H.

Mycology and Plant Pathology Laboratory, Department of P.G. Studies and Research in Botany,
Gulbarga University, Kalburgi-585106, Karnataka, India

*Author for Correspondence

ABSTRACT

Pongamia pinnata has been found to be one of the most suitable bio-fuel plants due to its various favorable attributes. Numbers of fungal species were found associated with seeds. Among these fungi *C. cladosporioides* was dominant followed by *Alternaria* and *Aspergillus* species. The aim of research work is to estimate primary metabolites such as total lipid, protein, reducing sugar, carbohydrate and starch which are present in seeds of *Pongamia pinnata*. The percent of total Lipid content in healthy seeds (0.79mg/gm), reducing sugar (1.26mg/gm), protein (2.66 mg/gm) and carbohydrate (15.44 mg/gm) respectively. During fungal infection biochemical changes occur in the seeds and the infected seeds shows reduction in total lipid content (0.58 mg/gm), proteins (1.95mg/gm), reducing sugars (0.819 mg/gm) and carbohydrates (12.70 mg/gm). The percent of reduction in total lipid about 0.21%, reducing sugar 0.44% and carbohydrate 2.74%. From 100 gm of healthy seeds 37 ml oil is obtained by Soxhlet apparatus, while in infected seeds (100gm) it was 32ml. There was a reduction of 5% of oil.

Keywords: *Pongamia Pinnata*, Fungi, Total Lipid, Protein, Carbohydrates, Reducing Sugars, Starch, Oil

INTRODUCTION

Among the many tree species, which can yield oil as a source of energy in the form of bio diesel, *Pongamia pinnata* has been found to be one of the most suitable species. *Pongamia pinnata* (L.) Pierre or its synonym *Millettia pinnata* is a small evergreen tree species belonging to the plant family *Fabaceae*. This legume tree species is widely distributed in various regions of the world like Australia, China, India and Pakistan (Kirtikar *et al.*, 1994).

The biofuel obtained from *P. pinnata* is used as a bio-diesel in industries (De and Bhattacharya, 1999; Azam *et al.*, 2005; Karmee and Chadha, 2005). India has shown positive signs towards renewable energy technologies and committed to the use of renewable sources to supplement its energy requirements (Doshi and Gopal, 2013). The *Pongamia pinnata* plant resource as the best plant material with higher productivity, as the quality of bio diesel is dependent on the specific ratio of saturated and unsaturated fatty acids in the oil (Ramos *et al.*, 2009).

This plant has been widely used as a source of animal fodder, fuel, manure, fish poison, timber and traditional medicine for a long time. The pongam seed oil has a bitter taste and considered as non edible oil. It is used as fuel for cooking and lamps, lubricant, tanning leather making soap and as illuminating oil. In many parts of the world, production of Karanja for industrial (biodiesel) and medicinal purposes is gaining popularity (Nagalakshmi *et al.*, 2011) particularly in Ayurvedic medicine in bronchitis, whooping cough, rheumatic joints and diabetes. Leaves used as a medicated bath for relieving rheumatic pain and for cleaning ulcer in gonorrhea (Pathak *et al.*, 2015).

The seed oil contains karanja (Pathak *et al.*, 2015). It is estimated that the large scale of cultivation of *P. pinnata* would fulfill the demands of bio-fuel for India (Punia *et al.*, 2006). In supporting the demand of new and renewable energy, the use of biomass-based biofuels are needed and several forest tree species can be utilized as a source of raw materials (Syamsuwida *et al.*, 2015). The tree is also a host plant for Lac insect.

The plant to grow in larger areas is high, but very less is known about the plant pathology and loss due to the infections. In this investigation, studied fungi associated with plant and biochemical changes in the primary metabolites and on oil extraction.

Research Article

MATERIALS AND METHODS

The work done was in the mycology and plant pathology laboratory, dept. of Botany, GUG. The survey was carried out to collect the fruit and seed samples of *Pongamia pinnata*. The fresh *Pongamia pinnata* fruits were collected which are healthy, infected as well as seedless fruits, collected from different locations of GUG campus.

Isolation of Fungi:

To study the fungal association, infected fruits and seeds were placed on moist sterilized blotter and incubated at 28C. The spore suspension was prepared from infected parts of seeds and fruits separately and spore concentration was adjusted to 10^3 spores/ml and inoculated on PDA and MRBA nutrient medium and incubated at 28C. After a period of 24 h different fungi seen growing, were sub-cultured using a scotch tape method and identified using manuals (Barnet and Hunter, 1972; Raper and Fennel, 1965).

Biochemical Estimation:

For the quantitative estimation different protocols were used. Healthy and infected seeds were collected and washed with distilled water, dried and powdered. The powder was used for analysis of total lipid (Bligh and Dyer method), protein (Lowry's method), reducing sugar (Nelson-Somogyi method), carbohydrate (Anthrone method) and starch (Anthrone method). Oil extraction of the *Pongamia pinnata* done by Soxhlet apparatus. The oil was then extracted with hexane in a Soxhlet apparatus for about 12 hours. The solvent was then removed using a rotary vacuum evaporator at reduced pressure and the percentage of oil was determined

RESULTS AND DISCUSSION

Pongamia pinnata is one of the further fuel plants. Its management from disease, stress related yield loss is one of the very important criteria. Very few reports are with respect to diseases of this plant and that of fungal diseases very limited information is available. The plants are rich sources of high value metabolites like total lipid, protein, reducing sugar, carbohydrates and starch etc. A primary metabolites is directly involved in the development, reproduction and normal growth (Sagwan *et al.*, 2010). The fungal association with *Pongamia* plant may be parasitic or saprophytic but in this study reports change in biochemical due to interaction of fungi in the plant parts. According to Jamaluddin *et al.*, (2005) *Aspergillus* species is predominant in the stored seeds of *Pongamia*. In Anonymous (1969) reported association of few fungi on *Pongamia* tree.

Pongamia pinnata seeds were evaluated quantitatively for the analysis of protein, lipid, reducing sugar, starch and carbohydrates. In the present study, Phytochemical screening of *Pongamia pinnata* evaluated and results shown in table 1, biochemical estimation of primary metabolites of seeds of *Pongamia pinnata* has been undertaken. The higher amount of plant lipid can be used as natural food colors, essential oils and spice oleoresins. With a strong foundation in research and development, plant lipids have developed products that work with diverse requirements, be it culinary, medicinal or cosmetic (Yadav and Tyagi, 2006). Lipid content of the healthy seeds was 0.79gm/gm. the infected seeds shown the decreases in the lipid content that is 0.58gm/gm. the percentage reduction of about 0.21%, Compared to earlier reports of Kumar *et al.*, (2007); Solmon Raju and Rao (2005); Natanam *et al.*, (1989), Nagaraj and Mukata (2004), it is very less. Since we used crude method of collection and isolation, the percentage of lipid contents was less. When lipid content in infected seed was compared to healthy there was reduction of 0.21%. There are no such reports of reduction in lipid contents due to fungal association.

Proteins are the primary components of living things. The presence of higher protein level in the plant points towards their possible increase food value or that a protein base bioactive compound could also be isolated in Future (Thomsen *et al.*, 1991). Protein content in *Pongamia* seeds was 2.66mg/gm that was reduced to 1.95mg/gm with the percent reduction in the protein content of the seed is 0.71%. Earlier reports of Duke (1983), presence of 17.4% protein in dry kernels. Scott *et al.*, (2008) reported deoiled cake of seed contain 30% of protein which is good supplement of cattle feed. Hence, it is very important to reduce fungal association with seed as it reduces protein content.

Table 1: Estimation of Primary Metabolites in Healthy and Infected Seeds of *Pongamia Pinnata* (L.) Pierre

Sl. No.	Metabolites	In Healthy	In Infected	Yield Loss in %
1	Total Lipid	0.79mg/gm	0.58mg/gm	0.21%
2	Protein	2.66mg/gm	1.95mg/gm	0.71%
3	Reducing Sugar	1.26mg/gm	0.819mg/gm	0.44%
4	Carbohydrate	15.44mg/gm	12.70mg/gm	2.74%
5	Starch	24.70mg/gm	18.2mg/gm	6.5%
6	Extraction of Oil	37ml/100gm	32ml/100gm	5%

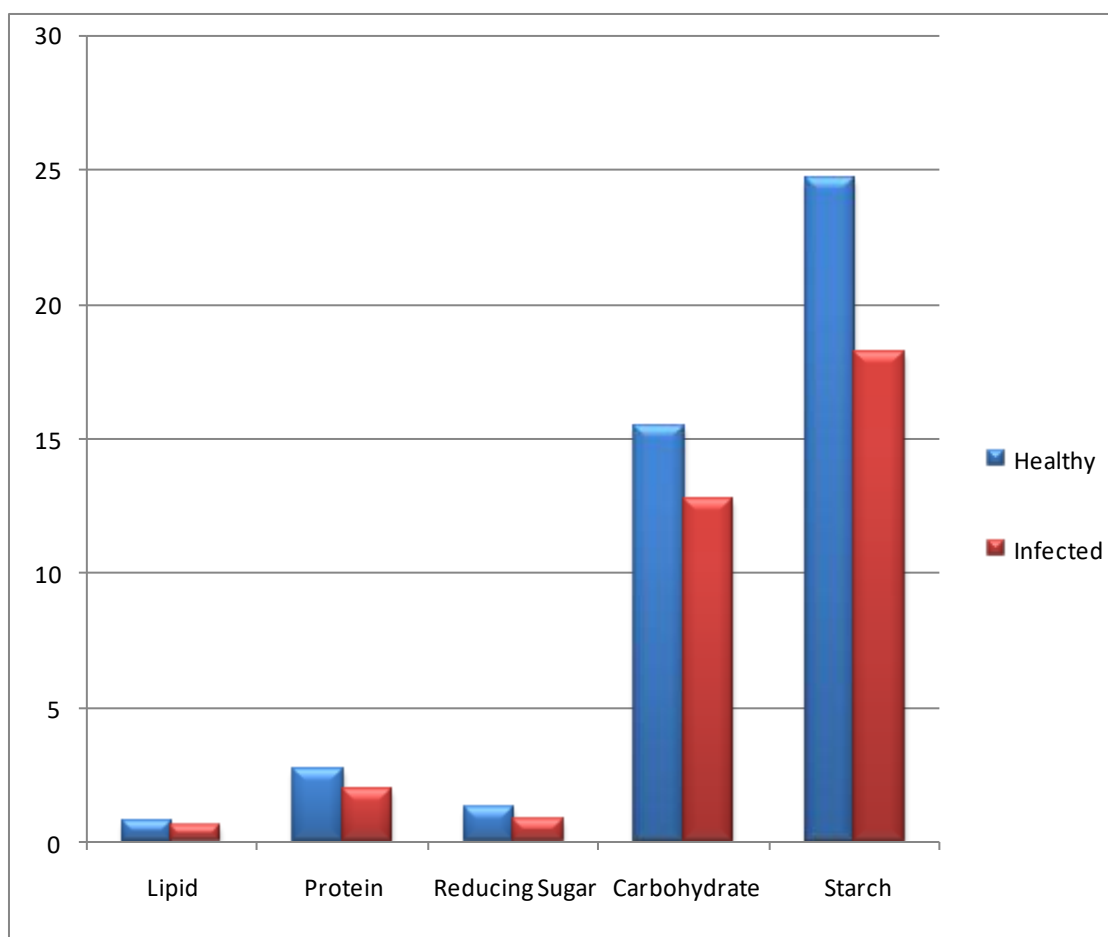


Figure 1: Concentration of Primary Metabolites of *Pongamia Pinnata* Seeds (mg/gm)

The reducing sugar in healthy seeds was 1.26mg/gm that was reduced to 0.819mg/gm in the infected seeds with the percentage reduction of 0.44%. Sugars are very important biochemical nutrients for the plants. If abundantly existing, these are detected naturally by various other micro-organisms like bacteria, fungi, insects etc (Srilakshmi, 2012). No reports are there with this kind of reductional information. Hence, this is one of the unique additions to the knowledge of *Pongamia*.

Total carbohydrate content of healthy seeds was 15.44mg/gm. compared to report of Duke (1983), starch content was 6.6% which is less due to fungal association carbohydrate content reduced to 12.70mg/gm with reduction of 2.74%. Hence, the study of fungal association is very relevant and is required.

Plant sugars can be used as artificial sweeteners and they can even help diabetics by supporting the body in its rebuilding (Birajdar *et al.*, 2011). Starch is biodegradable and renewable in nature. They are

Research Article

increasingly being considered as an eco-friendly alternative to the use of synthetic additives in many other products, including plastics, detergents, pharmaceutical tablets, pesticides, cosmetics and even oil-drilling fluids (Sagwan *et al.*, 2010). The starch in healthy seeds was 24.70mg/gm and in a infected seeds 18.2mg/gm, with the total reduction is 6.5%.

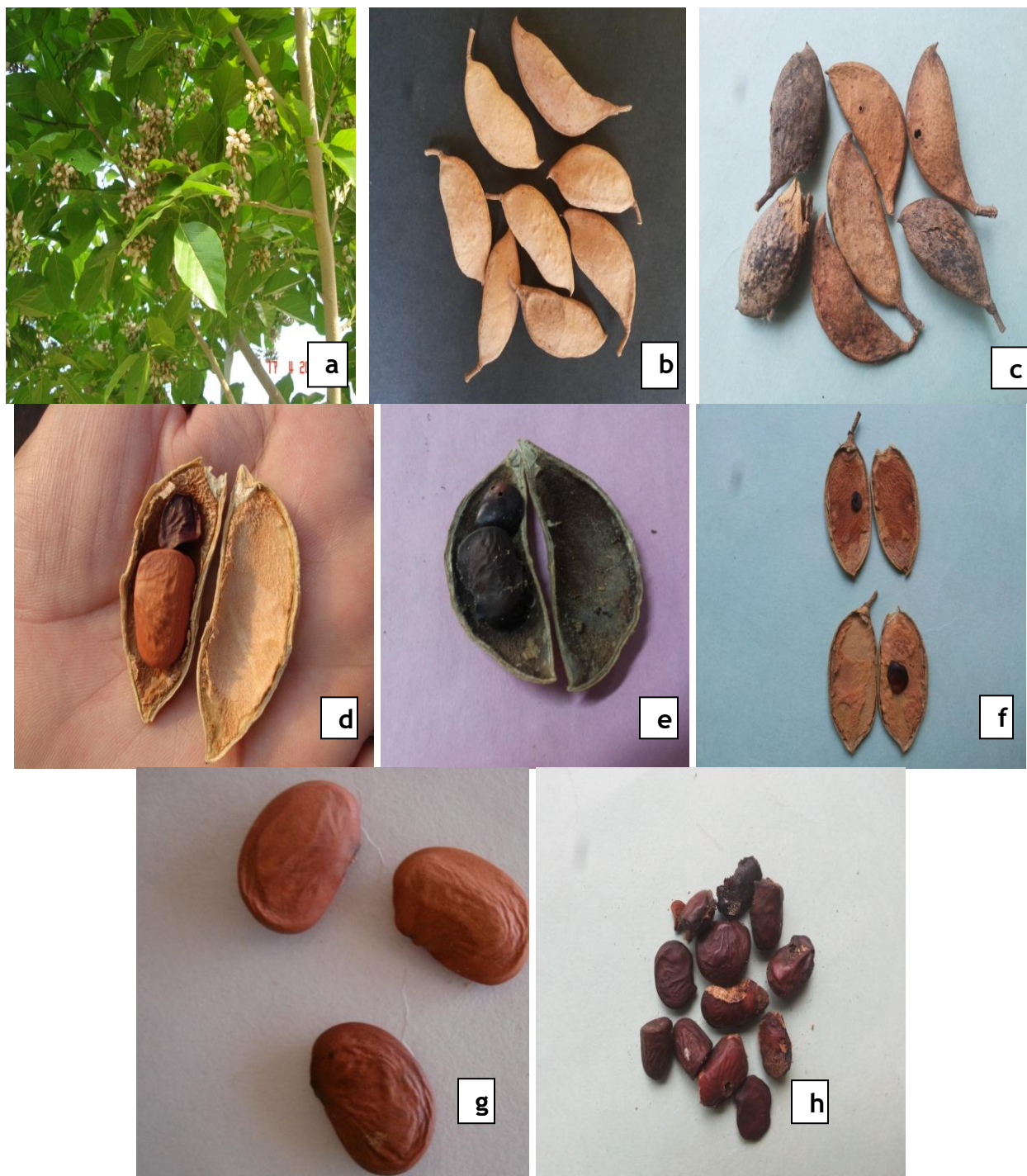


Figure 1: a- Healthy Plant of *Pongamia Pinnata*, b- Healthy Fruits, c- Infected Fruits, d- Healthy Fruits with seeds, e- Infected Fruits with Seeds, f-Seedless Fruits, g- Healthy Seeds and h- Infected Seeds

Research Article

Estimation of Oil:

Phytochemically the extraction of oil was carried out by using Soxhlet apparatus. The total content of oil from 100gm of healthy seeds after 65hrs. of evaporation was 37ml. where as in 100gm of infected seeds was 32ml. there was reduction of 5% of oil. The seeds contain about 27-39% oil and in the study of Kesari *et al.*, (2008), the highest oil yield 33% from seeds was recovered in n-Hexane. Seeds consist of 95 per cent kernel and are reported to contain about 27 per cent oil. The oil yield is reported to be about 24 to 26.5 per cent if mechanical expellers are used for the recovery of oil from kernels, but it is only 18-22 per cent from village crushers. The crude oil is yellow orange to brown in colour which deepens on standing. It has a bitter taste and disagreeable odour, thus, it is not considered for edible purpose.

We quantify that *Pongamia pinnata* contain many primary metabolites like carbohydrates, proteins, total lipids, reducing sugar and starch. These results are suggestive of primary bioactive compound of commercially importance and may result in great interest in plants pharmaceuticals. Primary metabolites analysis is necessary for knowing the nutritional potential of plants and them also from the precursors for the synthesis of secondary metabolites.

The plant pathogen causes significant losses in forests. Fungal pathogens are one of the most common resistant types of pathogens as well as prevent their infection is of utmost importance. Further studies are required with specific fungal disease and reduction in biochemicals. There is need to increase the productivity by increasing our basic knowledge especially by using seeds from the superior plants, possessing characteristics like high yield and high oil content.

ACKNOWLEDGEMENT

Author thankful to the Gulbarga University, Kalburgi for providing facilities.

REFERENCES

- Anynomus (1969).** *The Wealth of India: a Dictionary of Raw Materials and Industrial Products*, **8**, (Publications & Information Directorate, Council of Scientific & Industrial Research (CSIR), New Delhi, India).
- Azam MM, Waris A and Nahar NM (2005).** Prospects and potential of fatty acid methyl esters of some nontraditional seed oils for use as biodiesel in India. *Biomass and Bioenergy* **1,2** 293–302.
- Barnett HL and Hunter BB (1972).** *Illustrated Genera of Imperfect Fungi*, (Burgess Publishing Company, Vancouver, WA, U.S.A).
- Birajdar S, Kedarnath, Chinkod V and Patil CS (2011).** Phytochemical screening and characterization of *Pongamia pinnata* (L.) seed oil. *International Journal of Pharmaceuticals Analysis* **3**(1) 17-20.
- Bligh EG and Dyer WJ (1959).** A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* **37**(8) 911-917.
- De BK and Battacharyya DK (1999).** Biodiesel from minor vegetable oils like karanja oil and nahor oil. *Lipid/Fett* **101** 404–406.
- Doshi P and Srivastava G (2013).** Sustainable approach to produce bioethanol from Karanja (*Pongamia pinnata*) oilseed residue. *Turkish Journal of Agriculture and Forestry* **37** 781-788.
- Dubois M, Gilles K, Hamilton JK, Rebers PA and Smith F (1951).** A colorimetric method for the determination of sugar. *Nature* **168** 167.
- Duke JA (1983).** *Handbook of Energy Crops*. Unpublished [online]. Available: www.hort.purdue.edu/newcrop/duke_energy/pongamia_pinnata.html.
- Hedge JE and Hofreiter BT (1962).** In: *Carbohydrate Chemistry* **17** (edition Whistler RL and Be Miller JN) (Academic Press, New York, USA).
- Jamaluddin, Dadwal VS and Soni KK (1985).** Fungicidal effect on mycoflora and oil contents of sal (*Shorea robusta*) and Karanj (*Pongamia pinnata*) seeds during storage. *Seed Research* **13**(2) 66.
- Karmee SJ and Chadha A (2005).** Preparation of biodiesel from crude oil of *Pongamia pinnata*. *Bioresource Technology* **96** 1425–1429.

- Kesari V, Krishnamachari A and Rangadan L (2008).** Systematic characterization and seed oil analysis in candidate plus trees of Biodiesel plant, *Pongamia pinnata*, (L.) *Annals of Applied Biology* **152** 397-404.
- Kirtikar KR and Basu BD (1994).** Indian Medicinal Plants. (Dehradun Publishers India) **1,2** 830–832.
- Kumar S, Radhamani J, Singh AK and Varaprasad KS (2007).** Germination and seed storage behaviour in *Pongamia pinnata* L. *Current Science* **93**(7) 910-911.
- Lowery OH, Rosebrough NJ, Farr AL and Randall RJ (1951).** Protein measurement with the Folin-phenol reagent. *The Journal of Biological Chemistry* **193** 265-275.
- Nagalakshmi D, Dhanalakshmi K and Himabindu D (2011).** Replacement of groundnut cake with sunflower and Karanj seed cakes on performance, nutrient utilisation, immune response and carcass characteristics in Nellore lambs. *Small Ruminant Research* **97** 12–20.
- Nagaraj G and Mukata N (2004).** Seed composition and fatty acid profile of some tree born oilseeds. *Journal of Oilseeds Research* **21** 117-120.
- Natanam R, Kadirvel R and Chandrasekaran D (1989).** Chemical composition of karanja (*Pongamia glabra* vent [*P.pinnata*] kernel and cake as animal feed. *Indian Journal of Animal Nutrition* **6** 270-273.
- Pathak H, Maru S, Satya HN and Silawat SC (2015).** Fungal Diseases of Trees in Forest Nurseries of Indore, India. *Journal of Plant Pathology & Microbiology* **6** 297.
- Punia MS, Kureel RS and Pandey A (2006).** Status and potential of tree borne oil seeds (TBO'S) in biofuel production of India. *Indian Journal of Agroforestry* **8** 80–86.
- Ramos MJ, Fernandez CM, Caras A, Roudriguez L and Perez A (2009).** Influence of fatty acid composition of raw materials on bio diesel properties. *Bioresource Technology* **100** 261-268.
- Raper KB and Fennell DI (1965).** *The Genus Aspergillus*, (William and Wilkins, Baltimore, USA) 491-542.
- Sadasivam S and Manickam A (1996).** *Biochemical Methods*, II edition, (New Age International (P) Limited Publishers, New Delhi, India).
- Sagwan S, Rao DV and Sharma RA (2010).** Biochemical estimation of primary metabolites from *Pongamia pinnata* (L.): An important biodiesel plant. *International Journal of Pharmaceutical Sciences Review and Research* **5** 146-149.
- Scott PT, Pregelj L, Ning C, Hadler JS, Michael A and Gresshoff PM (2008).** *Pongamia pinnata*: An untrapped Resource for the Biofuels Industry of future. *Bioenergy Research* **1** 2-11.
- Solomon Raju AJ and Rao SP (2005).** Explosive pollen release and pollination as a function of nectar feeding activity of certain bees in the Biodiesel plant *Pongamia pinnata*(L.)Pierre (Fabaceae). *Current Science* **90**.
- Somogyi M (1952).** Note on sugar estimation. *The Journal of Biological Chemistry* **200** 245.
- Srilakshmi P, Sailaja D, Bhanuteja M, Kumar DR and Madhuri M (2012).** Quantitative estimation of Carbohydrate in insect induced and fungal infected leaf galls of *Pongamia pinnata*. *International Journal of Plant, Animal and Environmental Sciences* **2** 203-205.
- Syamsuwidada D, Putria KP, Kurniatya R and Aminahb A (2015).** Seeds and Seedlings Production of Bioenergy Tree Species Malapari (*Pongamia pinnata* (L.)Pierre). *Energy Procedia* **65** 67 – 75.
- Thomsen S, Handen HS and Nyman V (1991).** Ribosome inhibiting proteins from in vitro cultures of phytolacea dodecandra. *Planta Medica* **57** 232-236.
- Yadav PR and Tyagi R (2006).** *Lipid Biotechnology*, (Discovery Publishing House-New Delhi, India) 89.