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AS Shah
Research Scholar, Faculty of
forestry SKUAST-K, Benhama
Watlar Ganderbal-191201(J
&K), India

KN Qaisar
Dean, Faculty of forestry
SKUAST-K, Benhama Watlar
Ganderbal-191201(J & K), India

MA Rather
Division of Floriculture and
Landscaping SKUAST-K,
Shalimar-190025 (J & K), India

Estimated significance of biochemical constituents in seeds and pods a reliable criteria for judging maturity of "*Gleditsia triacanthos* variety inermis"

AS Shah, KN Qaisar and MA Rather

Abstract

Honey locust an important fast growing introduced multipurpose tree species of Kashmir. The tree can be adapted to environmental extremes such as drought, air pollutants and high light intensities. The propagation of this species not only ensures availability of fuelwood, fodder and timber supply but at the same time results in optimum use of degraded sites. Since under valley conditions, there was no documented information with respect to its biochemical maturity indices despite its immense potential for which the species is reputed throughout the temperate world. The present investigation was carried out in the faculty of forestry skuast-kashmir Shalimar, Srinagar during the year 2009 and 2010. The seeds and pods of *Gleditsia triacanthos* variety Inermis were collected from mid of July to first fortnight of November at an interval of 15 days and were subjected to determination of total sugar percentage, germination percentage and starch percentage. The chemical parameters with respect to total sugars and starch percentage data revealed that the total sugars in the seeds was 8.21 percent at first collection interval (15th July) which increased gradually with subsequent collection intervals to maximum value of total sugars 27.08 percent at the 6th collection interval (1st October). The corresponding values of germination percentage at the collection interval (6th collection) when the values of total sugars 27.08 percent was found maximum recorded 65.83 percent highest germination percentage. Similarly the starch percentage level showed the same trend increased with the advancement of collection dates from 22.11 percent to 38.32 percent from 1st to 5th collection interval respectively, which were statistically found highly significant. The chemical maturity indices of pods with respect to total sugars and starch percentage varied significantly. The mean values of two years data revealed that the total sugars increased from 11.21 percent, at first collection interval on 15th July to 6th collection interval on 1st October with value 30.18 percent. Moreover, the starch percentage in pods of *Gleditsia triacanthos* variety Inermis was recorded as the starch percentage in pods on 1st collection interval (15th July) was found 23.11 percent, which was at par with 2nd collection (1st August) 25.32 percent, however, there was gradually increase in starch percentage with the advancement of further collection interval dates up to 5th collection (15th September) 44.15 percent. Thus the biochemical analysis of present study with respect to determination of total sugars, germination percentage and starch content in seeds and pods of *Gleditsia triacanthos* variety Inermis depicted the authenticated judgement of maturity as a reliable criterion to be depending on.

Keywords: biochemical properties, *Gleditsia* spp., total sugars, starch concentration, germination percentage, seeds and pods.

Introduction

Forests occupy a place of considerable importance in the economy of the Jammu and Kashmir State and are more popularly known as green gold. The average income generate from the forest of Jammu and Kashmir is about 4269.01 lakhs (Anonymous, 2012) [5]. They restore ecological balance of all ecosystems, maintain biological diversity, act as catchments for soil and water conservation and prevent floods also. The state of Jammu and Kashmir is very famous all over the world for its lofty mountains, fascinating valleys, lakes, streams and lush green forests. The State is located in the north-western extremity of India between 32°-17' and 38°-58' north latitude and 73°-35' and 80°-36' east longitude with an average altitude of 1,586 metres from the mean sea level and annual precipitation of about 794.7 mm. From north to south, it extends over 640 kilometres in length and from east to west over 480 kilometres in breadth (Anonymous, 2003) [1]. The total geographical area of Jammu and Kashmir inside LOC is 10.138 million hectare, which constitutes about 6.74 per cent of the total area of the country. The total forest area of Jammu and Kashmir is 2.023 million hectare (Anonymous, 2011) [4], which contribute 19.95 per cent of total geographical area. Of the geographical area of 10.138 million hectares inside LOC, 62.70 per cent (6.358 million hectare) is under permanent snow cover, glaciers and cold deserts. Potential land resources of Jammu and

Correspondence

AS Shah
Research Scholar, Faculty of
forestry SKUAST-K, Benhama
Watlar Ganderbal-191201(J
&K), India

Kashmir have thus been constricted to 3.781 million hectare only. Dense forest tree cover of Kashmir valley is 0.3296 million hectares (21.03 % of its geographical area). Degraded forest have engulfed over 0.4774 million hectare. In Ladakh region, forest cover is 0.21 per cent only. Similarly in Jammu division, dense forest cover exist on 0.576 million hectare (23.24 % of its geographical area). In whole state 1.0765 million hectare of demarcated forests are degraded. Waste lands in Jammu and Kashmir are spread over an area of 0.946 million hectare which include 0.262 million hectare in vale of Kashmir, 0.557 million hectare in Jammu division and 0.127 million hectare in Ladakh region (Anonymous, 2011) [4].

The Kashmir Himalaya, known for its indigenous and endemic flora, also provides home to a large number of exotic plants, which exhibit a wide taxonomical and distributional stretch. The inventorization and documentation of its exotic ornamentals has received a little or negligible attention. There is a lot of scope for these exotic ornamentals in the Floriculture and Forestry industry of Kashmir, but lack of authentic identification has been as big hurdle in their scientific management. Exotic ornamentals grown in Kashmir show better performance in both quality and quantity attributes as compared to other regions of India (John *et al.*, 2007).

The valley of Kashmir is witnessing continuous introduction of exotic plant and animal species. The high-tech cross border transport and migration of humans has provided easy pathway to plant species to witness a wide spread on the world map. Kashmir valley grows 317 exotic ornamental plant species, which belong to 209 genera and 85 families. Dicotyledons are the largest group, represented by 252 species in 163 genera and 67 families. Monocotyledons comprise 52 species in 37 genera and 13 families. Gymnosperms are the smallest group, with 13 species distributed in 9 genera and 5 families. Asteraceae is the largest family containing 33 exotic ornamental species (Shabana *et al.*, 2010) [24].

Gleditsia spp. is deciduous a multipurpose tree species (MPTS) that is useful for wind breaks, shelter belts, erosion control, wildlife food and local wood products (posts and rail road ties) (Blair, 1990) [7]. Honey locust is used for buffer strips along highways or in urban forests, where it can be successfully grown in areas with air pollution, poor drainage, salty soils and drought (Ertekin and Kirdar, 2010) [16]. Honey locust has also become highly valued as an agroforestry species in other parts of the world (Davies and MacFarlane, 1979) [13].

Gleditsia spp. (Honey locust) is a valuable legume tree is well suited for cooler climates. It is very tolerant to lower temperatures ranging from 15 to 24 °C. The honey locust has deep tap roots and can survive on all but the driest soil, good growth occurs with 500 – 1500 mm of rainfall. It can be successfully grown to elevations of 1,500 meters in temperate areas and has survived at 2,500 metres in subtropical highlands. In addition to fuelwood, this species produces pods with a sweet tasting pulp that are edible by people. The pods can also be used to make high quality feed for animals (Anonymous, 2005) [2]. Honey locust is a shade intolerant tree, and will only become established in openings. It has a strong taproot and profusely branched root system. Its best growth in the United States is found on deep soils (pH 6.0 to 8.0) in moist, alluvial flood-plains between 35 and 40°N latitude (Blair, 1990) [7]. *Gleditsia* spp. family Leguminosae (subfamily – Caesalpinoideae), attains a normal height of 15–25 m and 0.51 m diameter (maximum height 50 m, diameter 1.8 m). Trees have a short bole and open, narrow or spreading

crown with reddish brown to black scaly ridged bark, often covered in clusters of large, branched thorns (Harlow *et al.*, 1996) [18].

Honey locust (*Gleditsia triacanthos* var. *inermis* Wild.) is thornless, or nearly so and slender in habit; bushy honey locust (*Gleditsia triacanthos* var. *elegantissima* [Grosdemangel Rehd.] is unarmed and densely bushy; Bujot honey locust (*Gleditsia triacanthos* var. *bujotii* [Neuml Rehd.]) has slender pendulous branches and narrow leaflets; and dwarf honey locust (*Gleditsia triacanthos* var. *nama* [Loud.] (Funk, 1965) [7].

Honey locust is found most commonly on soils in the orders Alfisols, Inceptisols and Mollisols that originate from limestone or the rich alluvial flood plains of major rivers and streams. Growth is poor on gravelly or heavy clay soils. Honey locust often fails on shallow soils. Although ample soil moisture is necessary for best growth. The species is very resistant to drought. Because of this, it is a valuable species for shelter belt planting in the Great plains (Funk, 1965) [7]. The species is also tolerant of acidic soils (Whitecomb, 1976) [26], but best development is usually on soils having a pH between 6.00 and 8.00 (Monk *et al.*, 1961) [22].

Gleditsia assamica only species of genus gleditsia reported from north eastern part of India. *Gleditsia assamica* grows in primary and secondary forests of north east India at an elevation range of 100-250 m altitude. It is a medium to large sized tree (15-20 m) with a conical crown and thick canopy. Bright green glossy leaves are alternate, pinnately or bipinnately compared, typically with many small leaf lets. Small flowers bloom during March to May. Pods are 20 to 50 x 1 cm in dimension, flattened and straight and mature during September to October. Spines are numerous during seedling stage while these are prominent only on trunk in mature trees. Pods have ethnobotanical use and garo tribes of Nokrek Biosphere Reserve use the paste to heal stomach ache. Clear felling along with habitat degradation severely affected the natural population of *Gleditsia assamica* and the species is also listed as vulnerable in the IUCN red list of threatened species (IUCN, 2009) [20].

Regeneration of *Gleditsia assamica* was found diminishing and only 11 mature trees were traced from Nokrek Biosphere Reserve. The density of the species was 2.75 ha⁻¹ but is restricted only to very small areas of the reserve. Seedling and sapling population was also scanty and contributed 57.45 and 19.15 per cent, respectively in the Nokrek Biosphere Reserve, Garo Hills, Meghalaya in the studied sites (Choudhury and Khan, 2010) [2]. The present investigation was executed with this aim to judge the maturity of “*Gleditsia triacanthos* var.*inermis* Wild.” through the biochemical examination method of its components.

Materials and Methods

The investigation conducted on “Estimated Significance of Biochemical Constituents in Seeds and Pods as a Reliable Criteria for Judging Maturity of *Gleditsia triacanthos* variety *Inermis* an Ornamental Plant” was carried out in the Faculty of Forestry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar during the year 2009 and 2010

2.1 Sampling Collection Intensity for Determining the Total Sugars, Starch and Germination Percentage Attributes in Seeds of *G. triacanthos* Var. *inermis* of the Year 2009.

The samples of seeds were collected from identified

phenotypic ally superior trees located at different sites in Srinagar district of Jammu and Kashmir and then were taken to laboratory in the faculty of forestry SKUAST-Kashmir during the year 2009. The collection of seed samples were started immediate after the seed setting in *G. triacanthos* Var. *inermis* tree, each after an interval of fifteen days. The 1st collection on (15 of July) of seed samples was initiated from superior trees. The sampling intensity were 200 seeds collected in each interval of fifteen days and were subjected to further involvement of necessary operations such as sorting of sound seeds from the collection stock, drying the seeds in hot air oven till the constant weight obtained and pulverised the seeds into feasible form as per demands of standard procedures for determination of total sugars and starch, simultaneously from the same collected sampling material in each interval a required amount of seeds was left for examining the germination percentage in seed germinator under laboratory conditions. The mean values of each collection interval for each attribute was obtained after replicating the pulverised seed samples by four times to arrive at exact biochemical value. The total number of sampling collection intervals during the entire experiment were eight and the same attributes were determined in each interval of collection dates repeatedly to the end of 8th collection (1st November). The determined mean values of total sugars, starch and germination percentage were presented in table-1 of the year 2009.

2.1.2 Sampling Collection Intensity for Deriving out the Total Sugars and Starch in Pods of *G. triacanthos* Var. *Inermis* of the Year 2009.

The pod samples were accumulated from identified superior trees located at various places in Srinagar district of Jammu and Kashmir for determination of total sugars and starch concentrations in them. The sampling material was packed in air tight polythene bags during the collection in the field. The packed sampling material was taken to laboratory of faculty of forestry SKUAST-Kashmir, for preparing the sampling material to further necessary operations involved therein in order to know the presence of total sugars and starch percentage concentrations in pods. The 100 number of pods were picked up from the sampling sources and were subjected to sorting out the disease free and sound pods in order to make correct estimation of attributes. Moreover, the sampling material was oven dried as per the recommendations of (ISTA 1993)^[19] standard, then the dried sampled pods were taken out from oven after obtaining their constant weight and were subjected to pulverise the sampling stock into completely powdered form, four replications of each sample made on purpose to arrive at the mean value of each attribute, during the analysis of samples. Similarly, all the collection intervals from 15th of July to 1ST November had been passed through the same procedure. The estimated concentration of total sugars and starch percentage mean values were presented in table-2 of the year 2009.

2.1.3 Sampling Collection Intensity for Determination of Total Sugars, Starch and Germination Percentage Attributes in Seeds of *G. triacanthos* Var. *inermis* of the Year 2010

The same practical procedure followed under this above highlighted heading which earlier has been brought about in 2.1 sub-heading for the estimation of identical attributes. The collection of samples started from the 15th of July to 1st November after every fifteen days of period. In each

collection interval 200 number of seeds were collected in small air tight polybags. The mean values of each attribute was obtained after replicating analysis of a sample by four times for its errant free reading and for correct statistical evaluation of the mean values. The statistically analysed mean values are presented in table-3 of the year 2010.

2.1.4 Sampling Collection Intensity for the Estimation of Total Sugars and Starch in Pods of *G. triacanthos* Var. *inermis* of the Year 2010.

Under this experimental heading, the same methodology brought in use which was earlier put in place for the same component of the estimation of total sugars and starch under sub-heading 2.1.2. The statistically observed mean values are presented in table-4 of the year 2010.

2.1.5 Pool Data Manifestation of Total Sugars, Starch and Germination Percentage Attributes In Seeds of the Year 2009 and 2010.

The determined mean values in seeds of the year 2009 presented in table-1 and the year 2010 presented in table-3 were combined and statistically analysed for interpreting the results of findings. The results of pool data of the year 2009 and 2010 are presented in table-5.

2.1.5.6 Pool Data Accumulation of Total Sugars and Starch Percentage Attributes in Pods of the Year 2009 and 2010.

The determined mean values in pods of the year 2009 presented in table-2 and the year 2010 presented in table-4 were combined and statistically analysed for interpreting the results of findings. The results of pool data of the year 2009 and 2010 are presented in table-6.

2.2 Methodology

The total sugars and starch of seeds and pods were determined by applying phenol-sulphuric acid method given by Dubios *et al* (1951)^[15] while as, germination percentage was obtained by calculating the total number of seeds germinated by the total number of seeds sown in all the replications and multiplied with 100 to arrive at the correct figure.

Results and Discussion

The chemical maturity indices of seeds of *Gleditsia triacanthos* variety *inermis* varied significantly (Table-5 & Fig. 1) over the collection periods due to the intense physiological activity occurs during the development of the seed/fruit, large quantity of soluble food being translocated into them and converted into new storage forms such as starch, fats and proteins and numerous other organic and inorganic constituents accumulates. In relevance to the present study of chemical maturity indices of seeds showed the same phenomenon occurred in terms of developing changes for storage of biochemical attributes in the seeds of subject species. The concentration of total sugars and starch gradually increased with the advancement towards the maturity. Hence it was observed that the total sugars and starch were 8.21 and 22.11 per cent at the first collection interval on 15th July, and no germination was recorded on that collection stage, respectively. Thereafter as long as total sugar showed gradual increase simultaneously germination was also recorded increased subsequently over the collection periods. Henceforth, the maximum total sugar, germination per cent were registered as 27.08 per

Table 1: Chemical maturity indices of seeds of *Gleditsia triacanthos* variety inermis for the year 2009

Collection intervals (15 days)	Total sugars (%)	Starch (%)	Germination (%)
1 st collection (15 th July)	8.20	22.09	0.00 (0.00)
2 nd collection (1 st August)	10.83	25.60	0.00 (0.00)
3 rd collection (15 th August)	13.90	28.32	16.04 (23.61)
4 th collection (1 st September)	17.68	34.80	29.60 (32.96)
5 th collection (15 th September)	22.80	38.30	45.89 (42.64)
6 th collection (1 st October)	27.06	33.51	65.80 (54.24)
7 th collection (15 th October)	27.00	29.30	55.20 (47.99)
8 th collection (1 st November)	27.00	26.18	40.77 (39.68)
CD(0.05)	1.82	2.94	1.35
SEm±	0.61	0.99	0.45

Figures in parenthesis are arc sine transformed values

Table 2: Chemical maturity indices of pods of *Gleditsia triacanthos* variety inermis for the year 2009

Collection intervals (15 days)	Total sugars (%)	Starch (%)
1 st collection (15 th July)	11.20	23.10
2 nd collection (1 st August)	13.80	25.30
3 rd collection (15 th August)	15.89	30.18
4 th collection (1 st September)	21.63	38.10
5 th collection (15 th September)	29.80	44.13
6 th collection (1 st October)	30.15	41.29
7 th collection (15 th October)	30.00	36.10
8 th collection (1 st November)	30.00	32.20
CD(0.05)	1.21	7.53
SEm±	0.41	2.54

Table 3: Chemical maturity indices of seeds of *Gleditsia triacanthos* variety inermis for the year 2010

Collection intervals (15 days)	Total sugars (%)	Starch (%)	Germination (%)
1 st collection (15 th July)	8.22	22.13	0.00 (0.00)
2 nd collection (1 st August)	10.87	25.64	0.00 (0.00)
3 rd collection (15 th August)	13.92	28.38	16.12 (23.67)
4 th collection (1 st September)	17.72	34.86	29.64 (32.98)
5 th collection (15 th September)	22.82	38.34	45.93 (42.67)
6 th collection (1 st October)	27.10	33.53	65.86 (54.25)
7 th collection (15 th October)	27.00	29.32	55.24 (48.01)
8 th collection (1 st November)	27.00	26.24	40.83 (39.72)
CD(0.05)	1.11	1.56	0.84
SEm±	0.37	0.52	0.29

Figures in parenthesis are arc sine transformed values

Table 4: Chemical maturity indices of pods of *Gleditsia triacanthos* variety inermis for the year 2010

Collection intervals (15 days)	Total sugars (%)	Starch (%)
1 st collection (15 th July)	11.22	23.12
2 nd collection (1 st August)	13.90	25.34
3 rd collection (15 th August)	15.93	30.24
4 th collection (1 st September)	21.73	38.14
5 th collection (15 th September)	29.82	44.17
6 th collection (1 st October)	30.21	41.35
7 th collection (15 th October)	30.00	36.24
8 th collection (1 st November)	30.00	32.24
CD(0.05)	1.72	1.44
SEm±	0.58	0.48

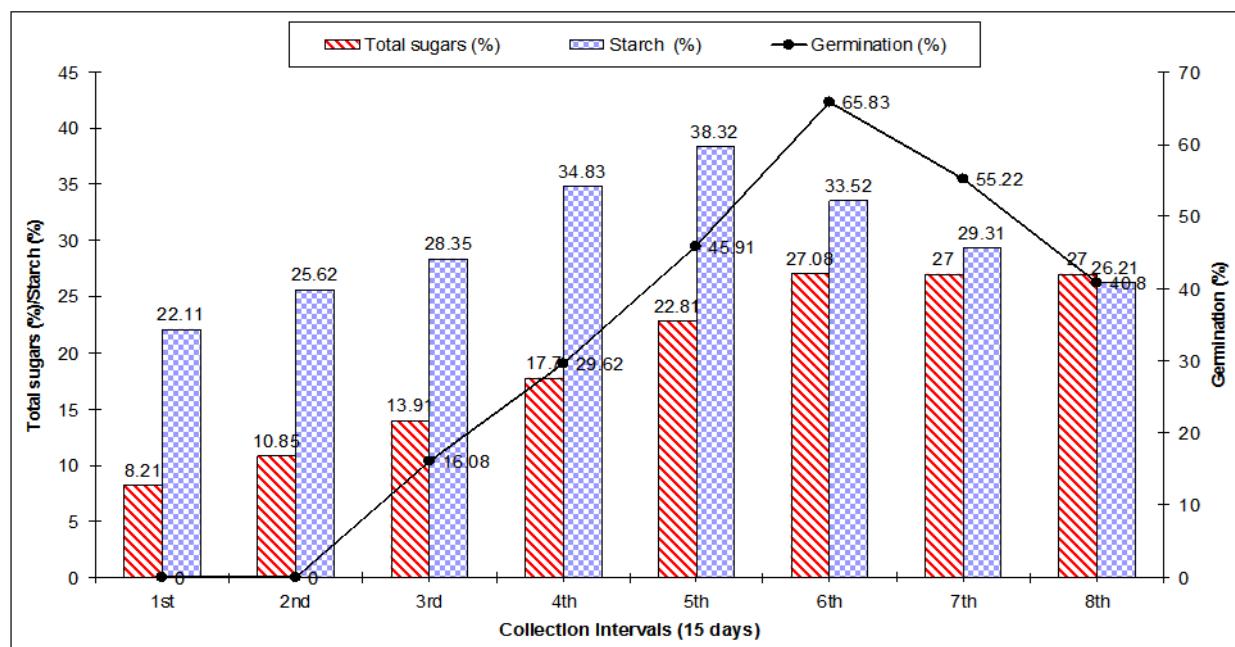
Table 5: Chemical maturity indices of seeds of *Gleditsia triacanthos* variety inermis (mean of two years data viz., 2009 and 2010)

Collection intervals (15 days)	Total sugar (%)	Starch (%)	Germination (%)
1 st collection (15 th July)	8.21	22.11	0.00 (0.00)*
2 nd collection (1 st August)	10.85	25.62	0.00 (0.00)
3 rd collection (15 th August)	13.91	28.35	16.08 (23.63)
4 th collection (1 st September)	17.70	34.83	29.62 (32.95)
5 th collection (15 th September)	22.81	38.32	45.91 (42.64)
6 th collection (1 st October)	27.08	33.52	65.83 (54.21)
7 th collection (15 th October)	27.00	29.31	55.22 (47.98)
8 th collection (1 st November)	27.00	26.21	40.80 (39.68)
CD(0.05)	1.54	1.50	0.90
SEm±	0.52	0.50	0.30

*Figures in parenthesis are arc sine transformed values

Table 6: Chemical maturity indices of pods of *Gleditsia triacanthos* variety inermis (mean of two years data viz., 2009 and 2010)

Collection intervals (15 days)	Total sugar (%)	Starch (%)
1 st collection (15 th July)	11.21	23.11
2 nd collection (1 st August)	13.85	25.32
3 rd collection (15 th August)	15.91	30.21
4 th collection (1 st September)	21.70	38.12
5 th collection (15 th September)	29.81	44.15
6 th collection (1 st October)	30.18	41.32
7 th collection (15 th October)	30.00	36.17
8 th collection (1 st November)	30.00	32.22
CD(0.05)	1.48	4.18
SEm±	0.50	1.41

**Fig 1:** Graphical representation of changes in total sugars, starch and germination per centage of seeds *Gleditsia triacanthos* variety inermis at different intervals

cent and 65.83 per cent on 1st October, respectively. After that declining trend was observed by the demonstrated attributes. Whereas, the starch maximum (38.32 %) on 15th September was observed earlier to sugar and maximum germination which indicates that conversion of starch into sugar for contributing maximum germination.

Maximum germination is related with maturity of the seed. It is an admitted fact and has been established with other forest tree species also seed germination per cent increased as the fruit matures. This is a result of accumulation of carbohydrates, fats and proteins in the seeds which proceed gradually across the season to maturity and is commonly defined by germinability as reported by Thapliyal and Gupta (1980) [25] in *Michelia champaca*.

Furthermore, the studies on fruit/seed biochemicals were reported in tree species by Bonner (1973) [9] who observed in most seeds, carbohydrates, fats, oils and proteins usually accumulate with maturity. Rediske (1961) [23] estimated seven biochemical constituents viz. crude fat, iodine absorption fat, reducing, non-reducing sugar, starch, soluble nitrogen and protein in *Pseudotsuga menziesii* seeds and correlated with maturity as determined by germination. Similar results were brought about by Dinesh (1990) [14] while working on *Celtis australis* found that the level of total sugars, starch and ether extract in seeds increased considerably, whereas, after attaining maturity starch and total sugars registered a minor decrease in their concentration. The findings of present investigation are in congruous with the outcomes of the

previous studies by Bonner (1973) [9], Rediske (1961) [23], Dinesh (1990) [14], Chauhan and Kumar (2002) [11]. Chauhan and Kumar (2002) [11] while working on *Acer oblongum* observed that total sugar and non-reducing sugar content of seeds increased over collection periods, whereas, reducing sugar content decreased with the advancement of capsule maturity.

The total sugars and starch per cent in the pods of honey locust showed considerably increasing and decreasing trend before and after the maturity of pods results presented in Table 6. Bonner (1974) [10] attributed the decrease in soluble carbohydrates to the conversion of carbohydrate into insoluble form during the period of dehydration in *Quercus nigra*, *Q. phellos* and *Q. falcata* acorns. On the other hand, it is so appeared that as the pods of honey locust matured, starch got converted to sugars, resulting in increase in total sugars throughout the study period. Similar study was carried out by Blanche *et al.* (1990) [8], who observed that starch content declined slowly in *Quercus nigra* with the advancement of maturity alongwith, there was no definite pattern of change in the contents of reducing and non-reducing sugars. These findings are in agreement with those of Bhupender *et al.* (2004) [6] in *Ziziphus mauritiana* and Blanche *et al.* (1990) [8] in *Quercus nigra* seeds.

Summary and Conclusion

The mean values of two years data viz; 2009 and 2010 showed increasing trend with the advancement of collection

intervals of seeds and pods, of *G.triacanthos var. inermis*. At the first collection interval on (15th of July), the total sugars, starch and germination percentage values in seeds and pods were recorded as 8.21 percent, 22.11 percent, 0.00 percent, 11.21 percent and 23.11 percent respectively. The mean values after the 1st collection on (15th of July) went on increasing with the seasonal advancement proceedings towards the maturity of subject species. The most maximum values were registered at 6th collection interval on (1st October) of both the components were recorded as 27.08 percent, 33.52 percent, 65.83 percent, 30.18 percent 41.32 percent respectively. Therefore it is pertinently concluded here that the significance of biochemical maturity indices of seeds and pods a dependable criteria for judging the maturity of *G.triacanthos* Var. *inermis* tree species.

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