CHAPTER - IV

FRUIT PHYSIOLOGY IN *SOLANUM VIARUM* DUNAL WITH SPECIAL EMPHASIS ON ANATOMICAL STUDY IN RELATION TO BIOSYNTHETIC SITE OF GLYCOALKALOID

INTRODUCTION

The physiological dynamics of the solasodine content during development of berry is a subject of great interest (Chauhan and Joshee, 1985). The amount of solasodine varies during berry development depending on the climatic conditions and the maturity of fruits. The observations on the growth and glycoalkaloid content of S. viarum Dunal has been described by Saini et al., (1965). The concentration maxima of solasodine was found to coincide with the fruit development (Correll et al., 1955 ; Sharma et al., 1979). The over ripened berries showed a decreased content of solasodine. Dynamics of solasodine biosynthesis and accumulation has been studied by Varghese et al., (1979). As the physiological variation of the content of solasodine depends on different biochemical parameters, attempts have been made to study the growth physiology of fruit according to its development with special emphasis on the estimation of different metabolites available in the tissue.

MATERIALS AND METHODS

MATERIALS :

Berries of S. viarum Dunal collected from the experimental garden of Centre for Life Sciences, North Bengal

University, have been utilized during the present investigation.

METHODS :

Berries of S. viarum Dunal at different ages (early developmental stage to brown stage of maturity) have been taken into consideration for the quantitative analysis of protein, soluble and insoluble carbohydrate, phenol, nitrogen (total), amino acids, chlorophylls and activity of different enzymes, polyphenol oxidase, catalase and peroxidase. Fresh sample have been used for the estimation of all the metabolites and enzymes activity. Freshly collected fruits were utilized after being fixed in 80% ethanol.

Quantitative estimation of carbohydrate (Mc Cready , et al., 1950).

50 mg samples of collected berries of different ages were crushed in 5 ml. of 80% ethanol and centrifused at 5,000 r.p.m. for 10 minutes. The supernatant was taken to estimate the soluble carbohydrate, while each residue was used for estimation of insoluble carbohydrate.

Estimation of soluble carbohydrate using the supernatent :

For each sample, the supernatent was taken in a beaker, and evaporated to dryness. It was washed with solvent ether and finally eluted with 80% ethanol. The volume was made upto 10 ml. To each of 3 ml. of elute 9 ml. anthrone reagent (0.1% in concentrated H_2SO_4) was added slowly under chilling condition and mixed well. Each of the mixture was kept in boiling water bath for 7-8 minutes and cooled immediately. The O.D. were measured at 610 nm in spectrophotometer (Model No. 106 Systronics).

Estimation of insoluble carbohydrate from the residue :

The residues obtained were digested separately with 25% H_2SO_4 (5ml) in water bath for 1 hr. at 100°C. They were cooled to room temparature and centrifused at 5,000 r.p.m. for 10 minutes. The standard curve for carbohydrates was prepared by using D-glucose (Glaxo) as the standard solution.

Estimation of total nitrogen (Vogel, 1961) :

Fresh sample of berries (50 mg) was taken for the estimation. It was digested with 1 ml. conc. H_2SO_4 for

an hour. The digested part was then dissolved in distilled water and filtered and the volume was made upto 100 ml. To 1 ml. of each solution, 1 ml. of 40% NaoH, 10% sodium silicate (1 : 1) 5 ml. freshly prepared Nessler's reagent were added. The yellow orange colour developed was measured in systronic spectrophotometer (Model No. 106) at 410 nm.

Preparation of reagent :

Nessler's reagent, 7.5 ml. of starch solution, 35 ml. of 10% NaOH solution and 7.5 ml. of distilled water were mixed. The resultant solution was ready for estimation. The standard curve for nitrogen was prepared using $(NH_4)_2$ So_4 solution as a standard solution.

Estimation of Chlorophyll (Arnon, 1949) :

To estimate chlorophyll (a & b) content of the fruits, [illegible] mg of fresh sample each from different ages of fruits were collected. Samples were placed in a glass morter and macerated to a paste with a small amount of 80% acetone. Maceration was continued with more acetone until the plant tissue became white. The acetone extract was centrifused at 5,000 r.p.m. for 10 minutes and each supernatent was taken

and diluted to 10 ml. with 80% acetone. The optical density of extract was determined separately at wave length of 649 & 665 nm. Total chlorophyll (a + b) per liter of extract was calculated. Total chlorophyll = A (a + b) mg. 1^{-1} = 6.5 x A 649 + 17.72 x A 665.

A 665 and A 649 stands for absorbant at 665 nm, and 649 nm respectively.

Quantitative determination of protein with Biuret reagent (Gornall et al., 1948).

50 mg of sample was taken from fruit of different stages and crushed separately with 0.05 (N) NaOH and the volume was made upto 5 ml. with water. Then each solution was centrifused at 5,000 r.p.m. for 10 minutes. The supernatent was taken and volume was made upto 10 ml. The control was made after mixing 5 ml. Biuret solution with 5 ml. dist. water.

Both the samples and the control were then kept in the incubator for at least 15 minutes at 37°C. It was then cooled to room temparature and the optical density was measured in spectrophotometer (Model No. 106), Systronics, at 520 nm. The ptotein content (mg/g fresh wt. basis) was measured following the standard curve with egg albumin.

800 mg NaOH crystal was taken in 100 ml. volumetric flask and dissolved in about 20 ml. of water. Then the ingradients of the Biuret reagent i.e., 900 mg Na-K-tertarate, 300 mg. $CuSo_4$, 500 mg. KI were dissolved it it successively and finally the volume was made upto 100 ml. with water.

Standard curve of protein was made using egg albumin (100 mg dissolved in 50 ml. water with a few drops of NaOH, 2.5% - 5% to facilate the solution).

Estimation of total amino acid content by ninhydrin reaction (Moore and Stein, 1948) :

50 mg of the fresh sample was taken from different ages of fruit separately for the estimation. They were crushed in boiled ethanol (80% v/v) immediately. The extracts, after suitable processing to remove extraneous materials present there in, were passed through resin columns (Dowex-50).

The extraction was followed by centrifugation of the samples at 5,000 r.p.m. for 10 minutes. The process was repeated twice and the ultimate volume of each of the

supernatent was made upto 100 ml. In each case, 5 ml. aliquot was mixed with an equal volume of ninhydrin solution (0.3% in 95% ethanol) and heated for 10 minutes at 80°C on water bath. They were allowed to cool to room temperature. The violet colour of the solution was determined in spectrophotometer (Model No. 106, Systronics) at 570 nm. Total amino acid (mg/g fresh weight basis) was measured following the standard curve of amino acid with DL - alanine.

For the preparation of 0.3% ninhydrin solution, 300 mg ninhydrin powder was dissolved in 20 ml. of 90% ethanol and finally the volume was made upto 100 ml. with abs. alcohol. The standard curve of amino acid was made using 1000 ppm. DL - alanine.

Estimation of total phenol (Swain & Hillis, 1959) :

50 mg of fresh tissue was taken. The extraction of phenolic acid was carried out following the method of Ibrahim & Tewers (1960). The blue colour developed with Folin-phenol reagent (Folin-Ciaealteau : water, 1 : 1) was determined in spectrophotometer (Model No. 106, Systronics) at 760 nm. The total phenol content (mg/g fresh wt. basis) was measured following the standard curve prepared with caffeic acid.

Extraction and estimation of Ribonuclic acid (Cherry, 1962 ; Markham, 1955) :

The extraction of RNA from the tissue was done following the method as described by Cherry (1962). 50 mg plant sample was homogenized in 5 ml. cold methanol and after centrifugation at 5,000 r.p.m. for 10 minutes, the supernatent was decanted off. The residue was washed successively with 0.2 M perchloric acid and absolute ethanol and then dissolved in 10 ml. of ethanol : ether (2:1, v/v) and kept at 50°C for 30 minutes. After centrifugation, the residue was dissolved in 10 ml. of 5% perchloric acid (v/v) and kept in a water bath at 70°C for 30 minutes and stored in a refrigerator for over night. The mixture was then centrifuged at 5,000 r.p.m. for 10 minutes and the supernatent was analysed for RNA. The content of RNA was determined according to the method Markham (1955). 3 ml. of orcinol reagent (1 g. of orcinol dissolved in 100 ml. of concentrated hydrochloric acid containing 0.1% $FeCl_3$) was taken in a boiling water bath for 20 minutes. After cooling down to room temparature the absorbance of the blue green colour was measured at 700 nm in spectrophotometer (Model No. 106 Systronic). The total RNA content (mg/g fresh wt. basis) was measured following the standard curve prepared with yeast RNA.

Extraction and estimation of catalase activity :

The extraction of the enzyme catalase and measurement of its activity were done according to the method followed by Biswas and Choudhury (1976).

The enzyme extract from 200 mg tissues was taken separately from different ages of fruit. The tissues were macerarated with chilled morter pastle in 3 ml. of 0.1 M phosphate buffer (pH-6.5) at $0^{o}C$. The homoginate was centrifused at 5,000 r.p.m. for 10 minutes. The supernatent was made upto 10 ml. with buffer and used as enzyme source.

The reaction mixture for the assay of the enzyme contained 1 ml. of the enzyme, 1 ml. of 0.0025 M H_2O_2 and the mixture was incubated for 5 minutes at $37^{o}C$. The reaction was stoped by adding 3 ml. of 0.1% Titanyl sulphate in 25% H_2So_4 and centrifused at 5,000 r.p.m. for 10 minutes. Then the O.D. of the colour was measured at 420 nm. Enzymatic activity was calculated following the method of Fick and Qualset (1975).

Extraction and Estimation of peroxidase activity :

The extraction of the enzyme peroxidase and

the measurement of its activity were done according to the method followed by Kar and Mishra (1976).

200 mg of the fresh tissues was homoginised in cold phosphate buffer 0.05 M (pH-6.5) and the volume was made upto 10 ml. with cold phosphate buffer 0.05 M (pH-6.5). The homogenised solution was centrifused at 10,000 g for 10 minutes and the filtrate was used as enzyme source.

In this case 5 ml. of assayed mixture contained 1 ml. of 50 µm H_2O_2, 300 µm of phosphate buffer (pH 6.8) 1 ml., of 15 um pyrogallol and 1 ml. of crude enzyme extract. After incubation at 25°C for 5 minutes the reaction was stopped with the addition of 1 ml. of 10% H_2SO_4 and the O.D. of the yellowish solution was measured at 430 nm. in spectrophotometer (Model No. 106, Systronic). The enzyme activity was measured following the method Fick & Qualset (1975).

Extraction and Estimation of Polyphenol oxidase :

The activity of the enzyme polyphenol oxidase was assayed following the method Kar and Mishra (1976).

200 mg tissues were homogenised in cold phosphate buffer 0.05 M (pH - 6.5) 10 ml. centrifused at 10,000g for 10 minutes separately. Filtrate was taken as enzyme source.

4 ml. of the assayed mixture contained 1 ml. 300 µm of phosphate buffer (pH - 6.8), 1 ml. of 50 um pyrogallol and 1 ml. of crude enzyme extract. After incubation at 25°C for exactly 5 minutes. The reaction was stopped with the addition of 1 ml. 10% H_2SO_4. Then the O.D. was measured in spectrophotometer (Model No. 106, Systronic) at 430 nm.

Quantitative determination of crude alkaloid (Gupta & Basu, 1981) :

S. viarum Dunal berries were oven dried (52°C) and powdered for estimation of crude alkaloid. 5 mg. dried powder was refluxed in hot methanol. This methanolic solution was treated as stock solution. From this, 0.5 ml. was mixed with 1 ml. of acetic resorcinol and H_2SO_4 for development of orange red colouration. The colour was stable after 5 minutes. The O.D. value was measured in spectrophotometer (Model No. 106, Systronics) at 510 nm. The percentage of crude alkaloid was calculated from the prepared standard curve using the solution of solasodine ranging from 10^{-6} ppm to 10^{-1}ppm.

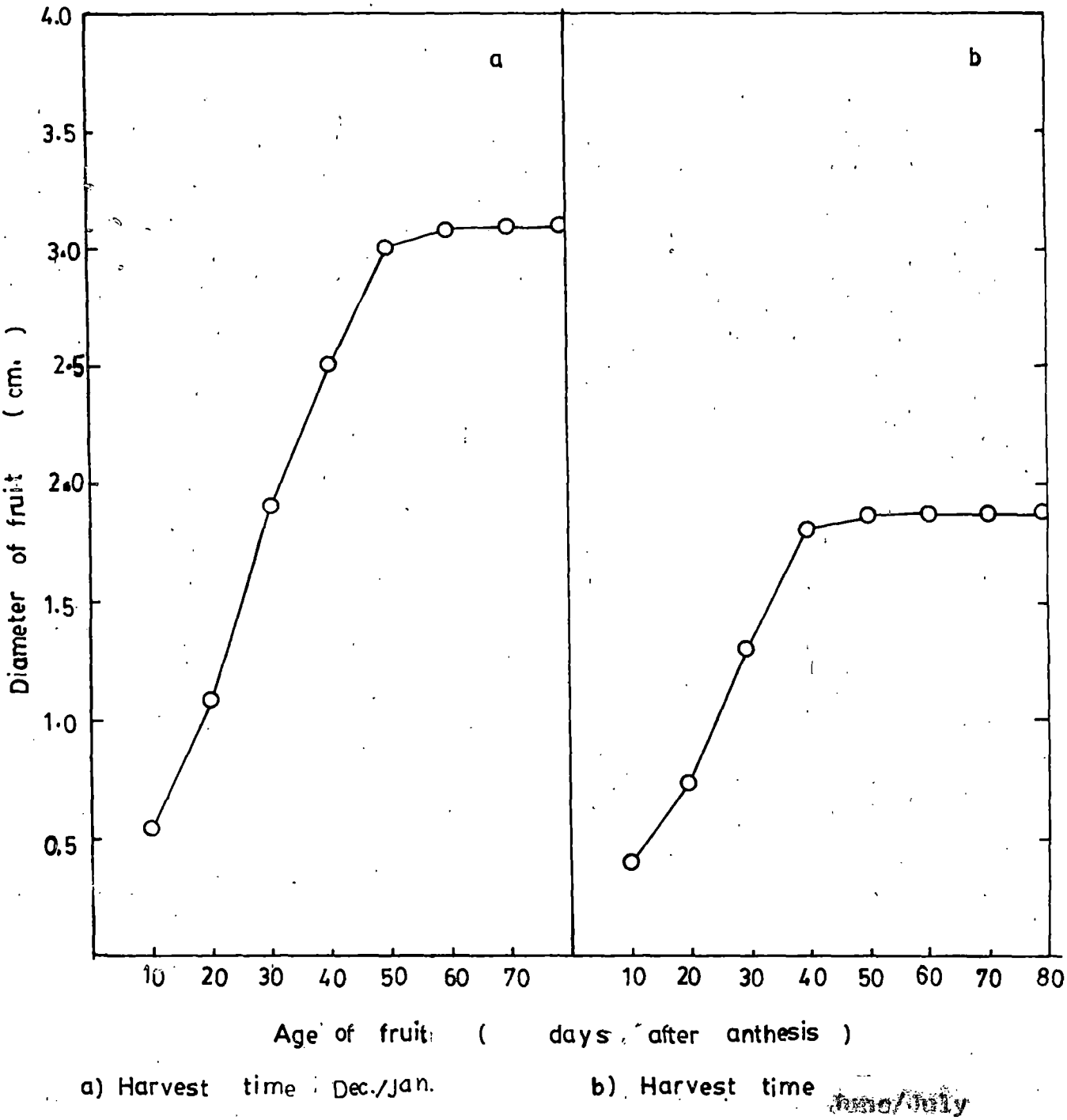

Fig. 15 : Growth of fruit of S. viarum at different season.

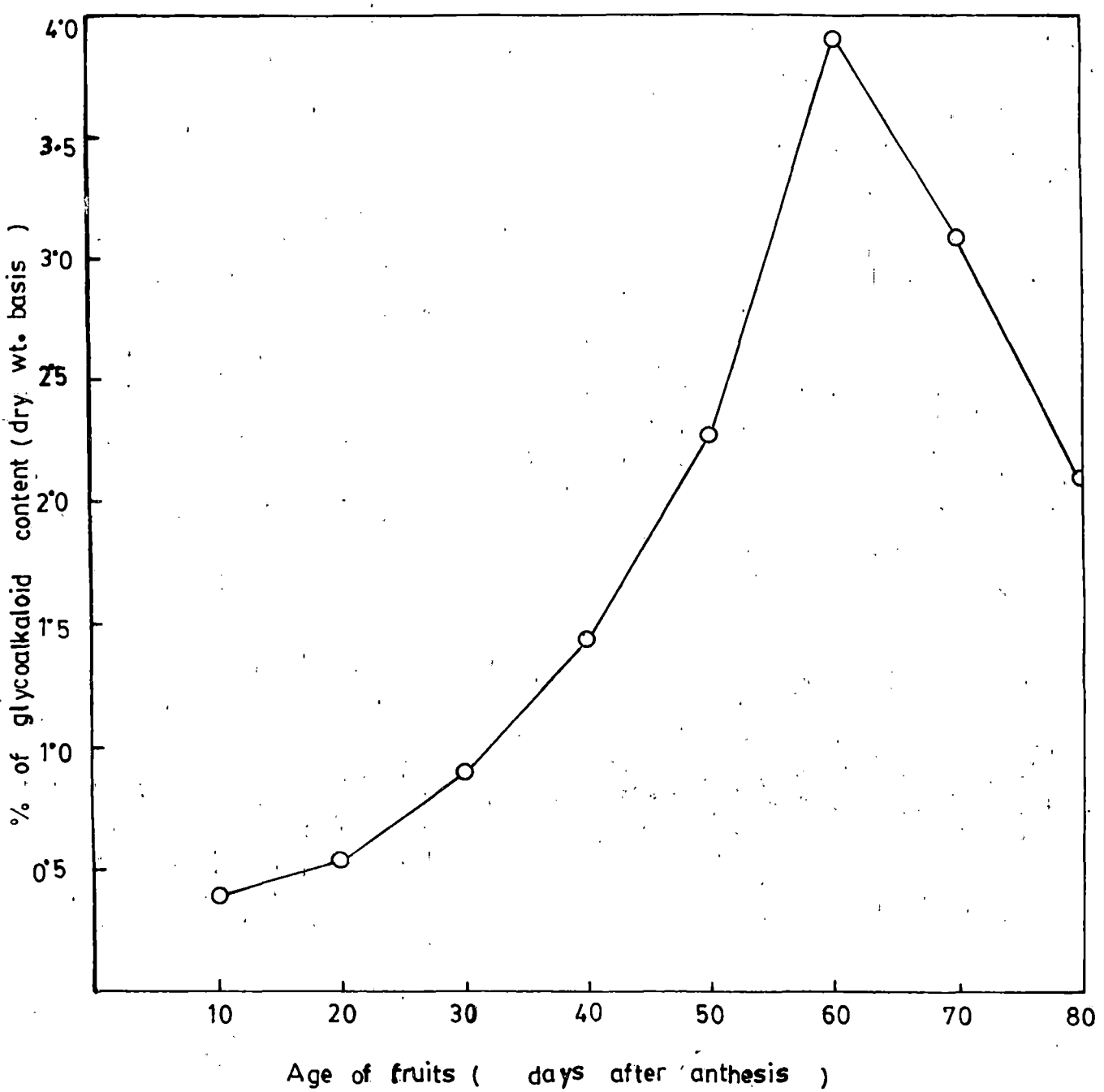

Fig. 16 : Glycoalkaloid content in developing fruit of S.viarum Dunal.

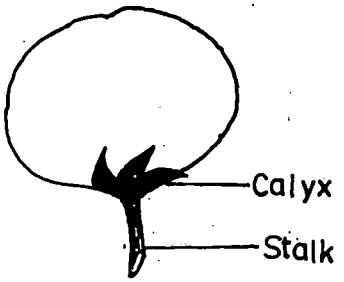

Fig. - 17a. Entire fruit (X 1)

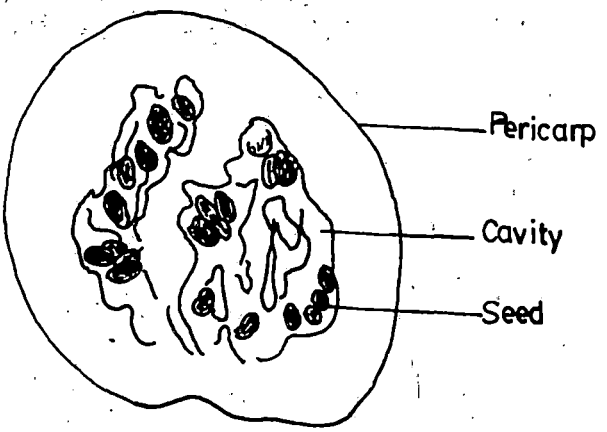

Fig. - 17b. L.S. of the fruit (X 3)

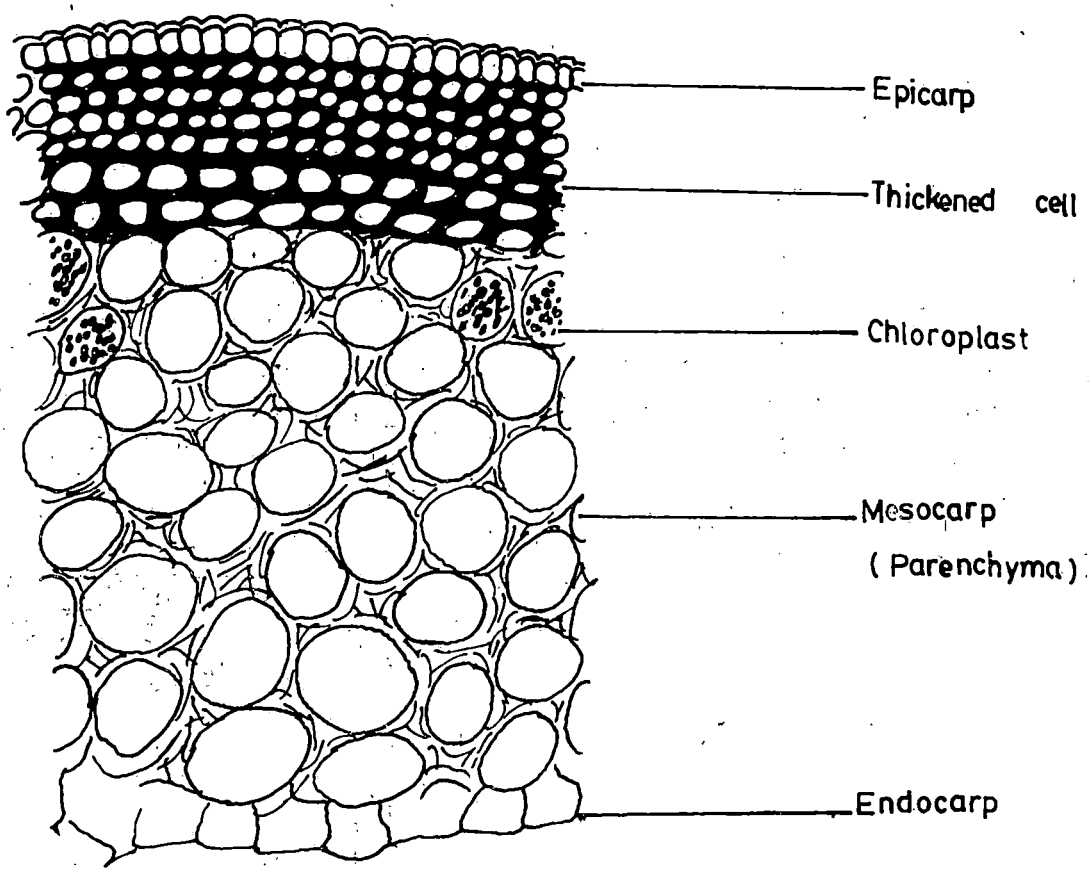

Fig. - 17c. L.S. of the pericarp (X 370)

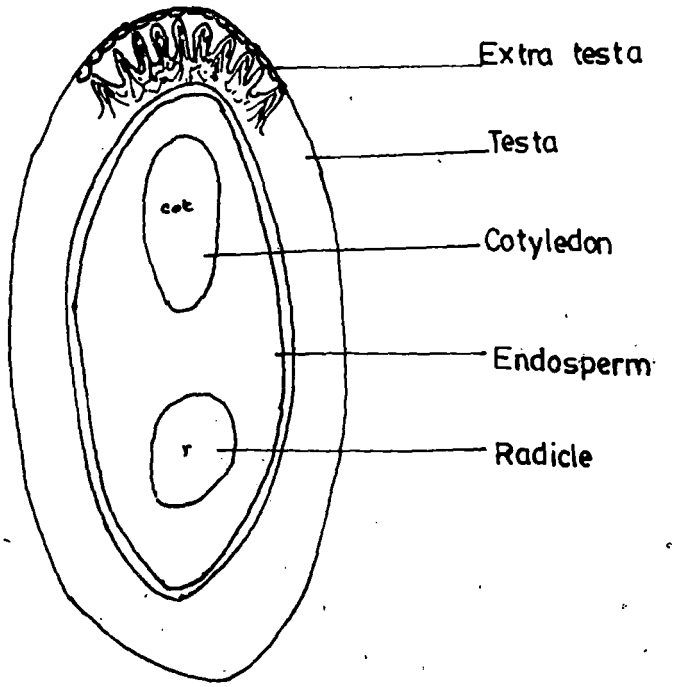

Fig. - 18a. L. S. of the seed (X 80)

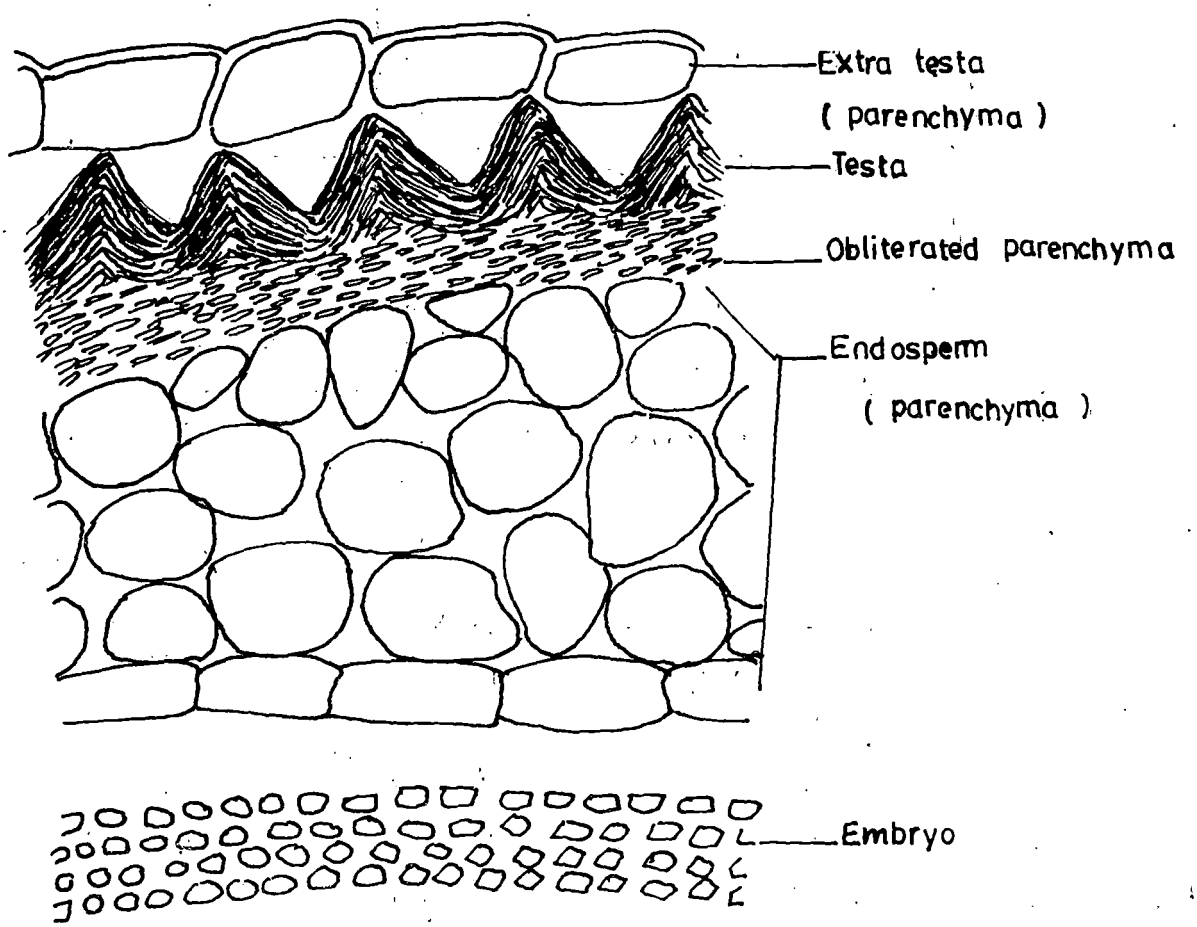

Fig. - 18b. L.S. of seed from 40 days old fruit (X 470)

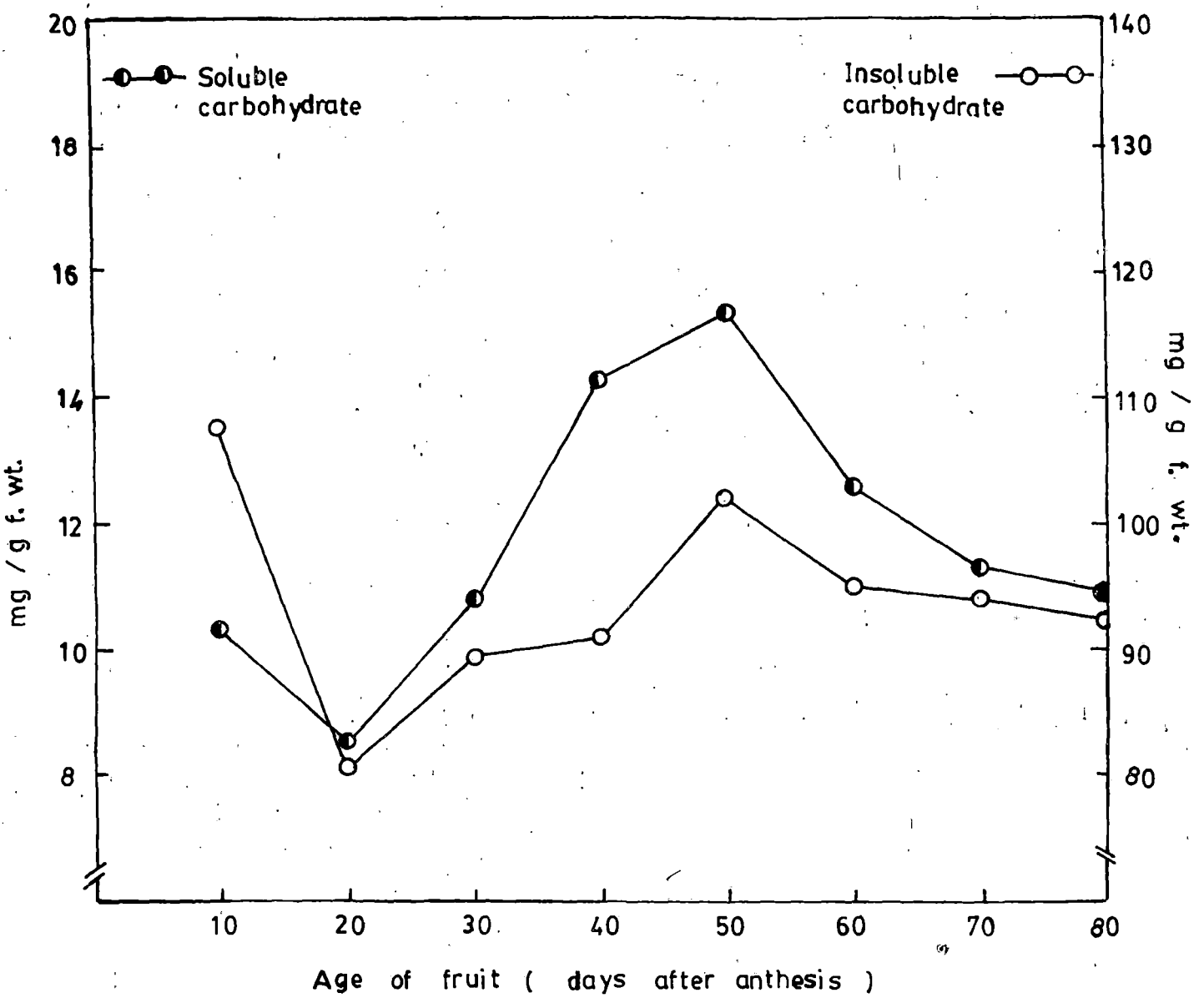

Fig. 19 : Soluble and insoluble carbohydrate content in developing fruit.

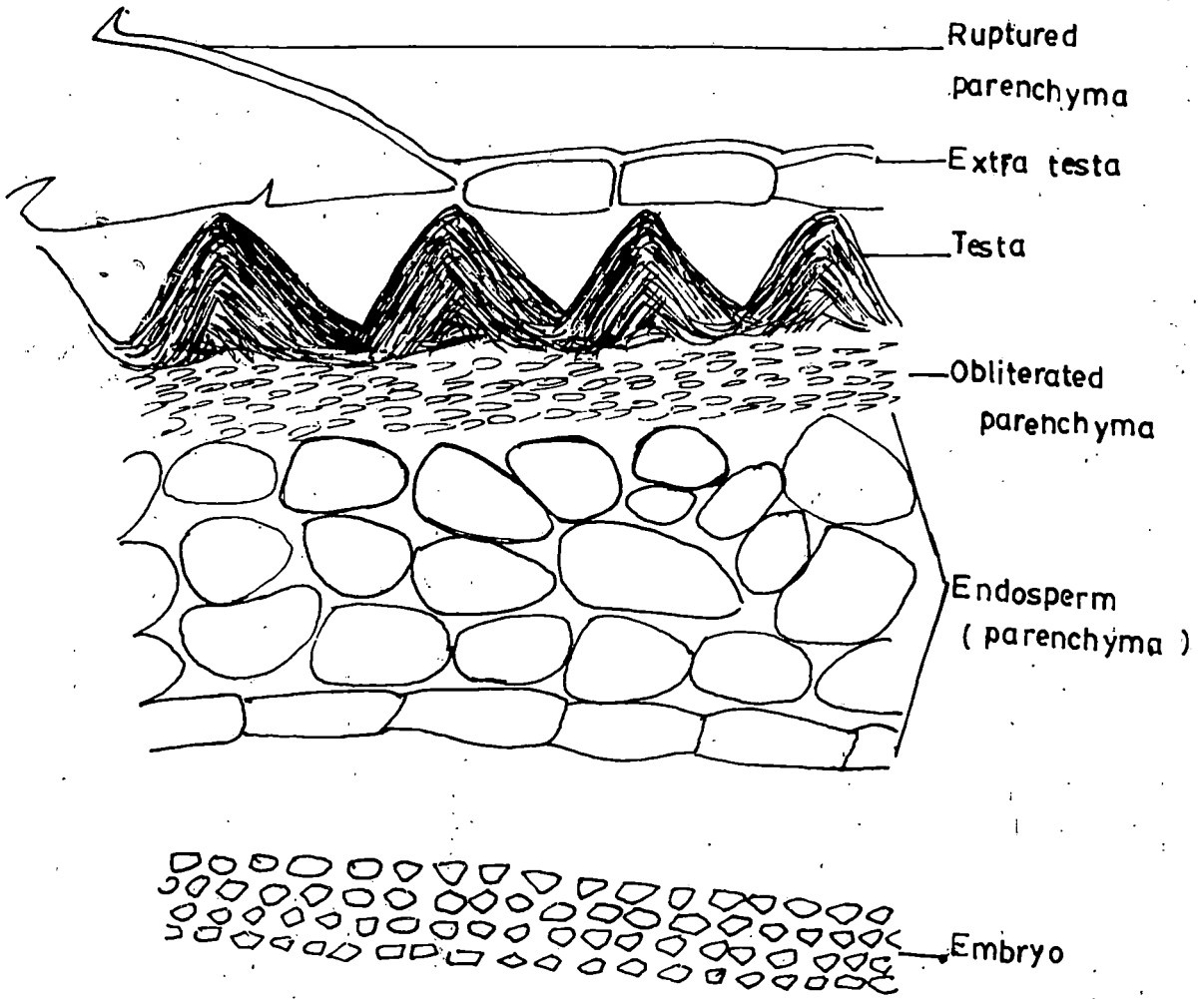

Fig. - 18c. L.S. of seed from 60 days old fruit (X 470)

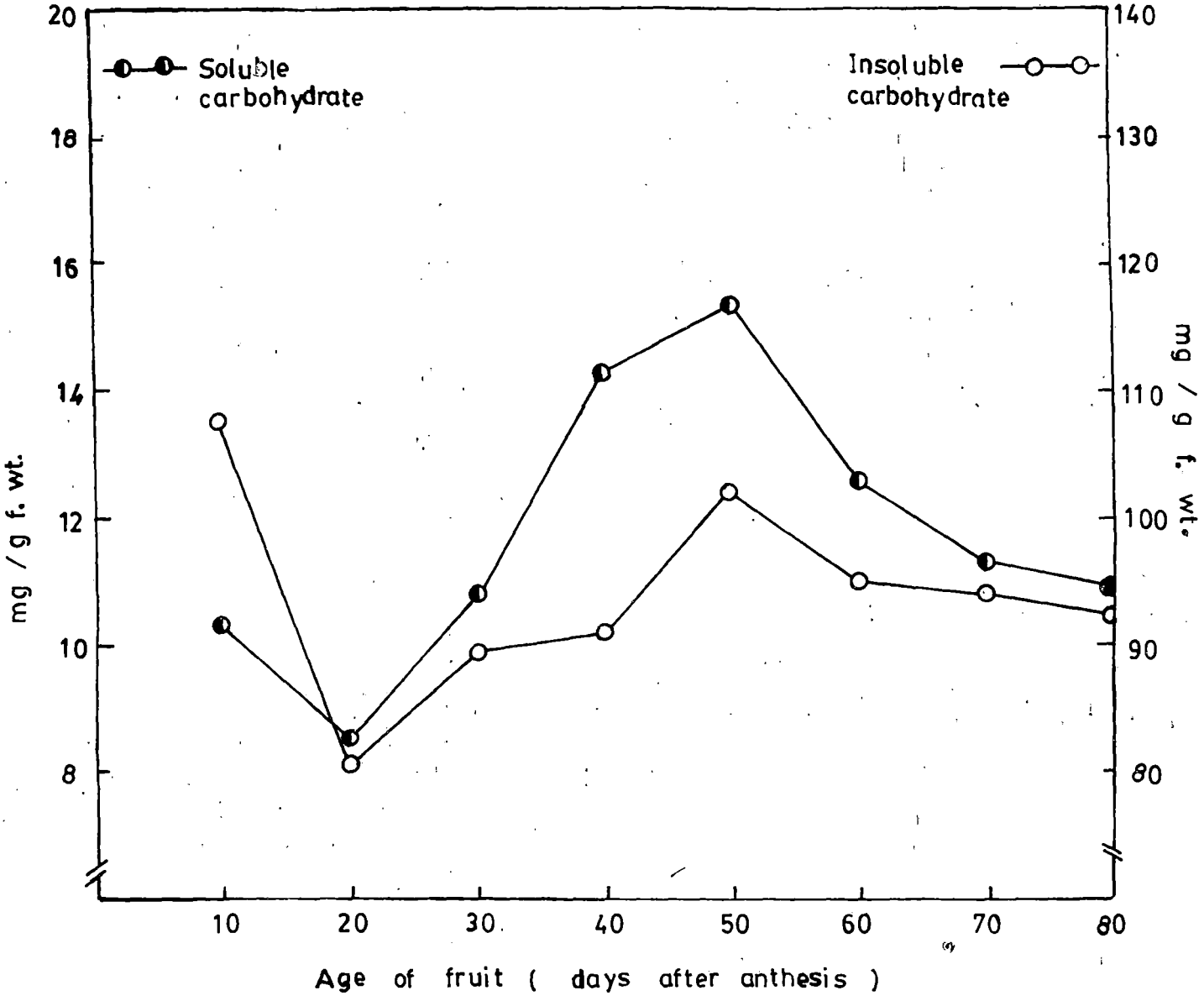

Fig. 19 : Soluble and insoluble carbohydrate content in developing fruit.

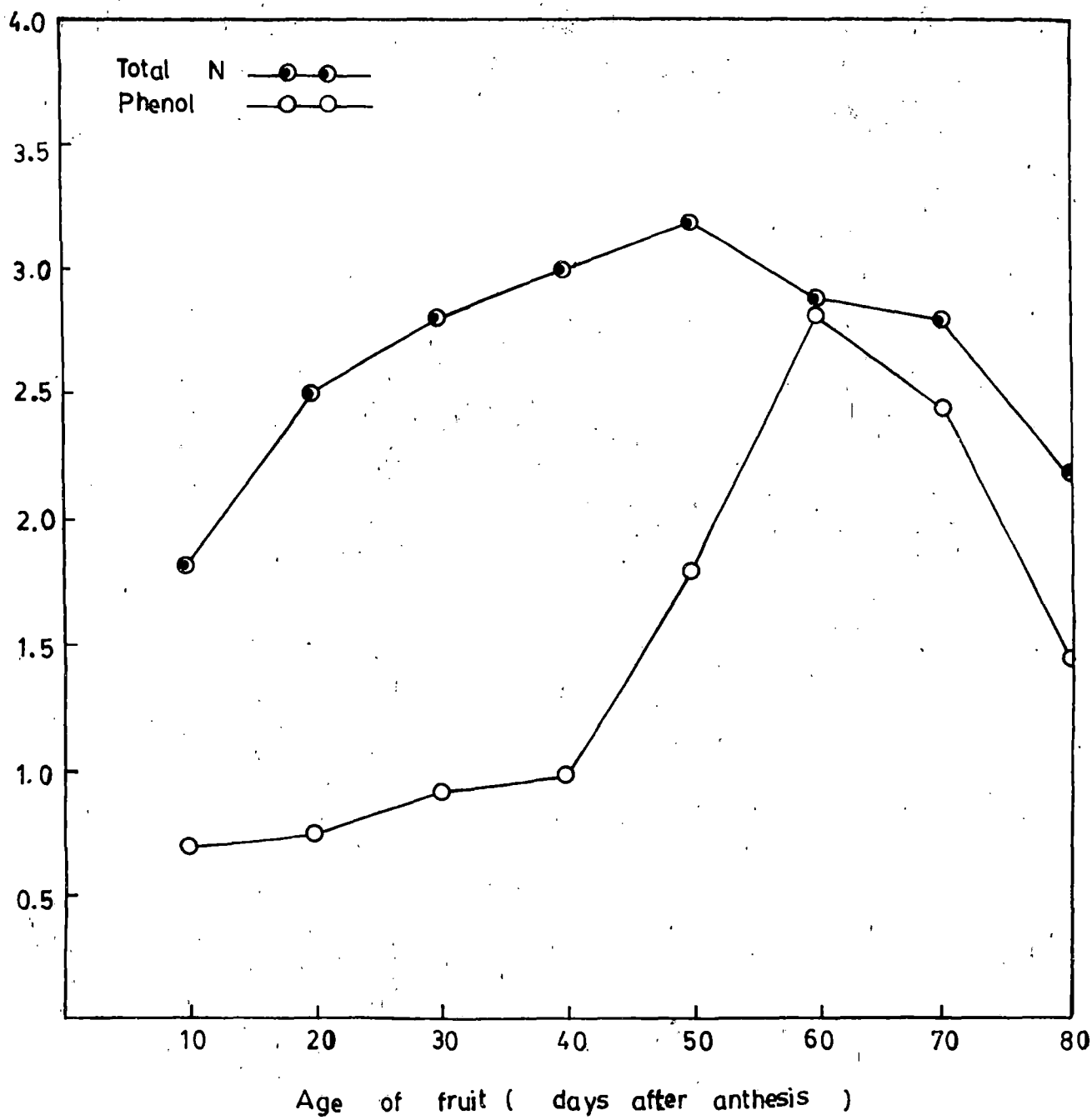

Fig. 20 : Total nitrogen and phenolic content in developing fruit of S.viarum Dunal.

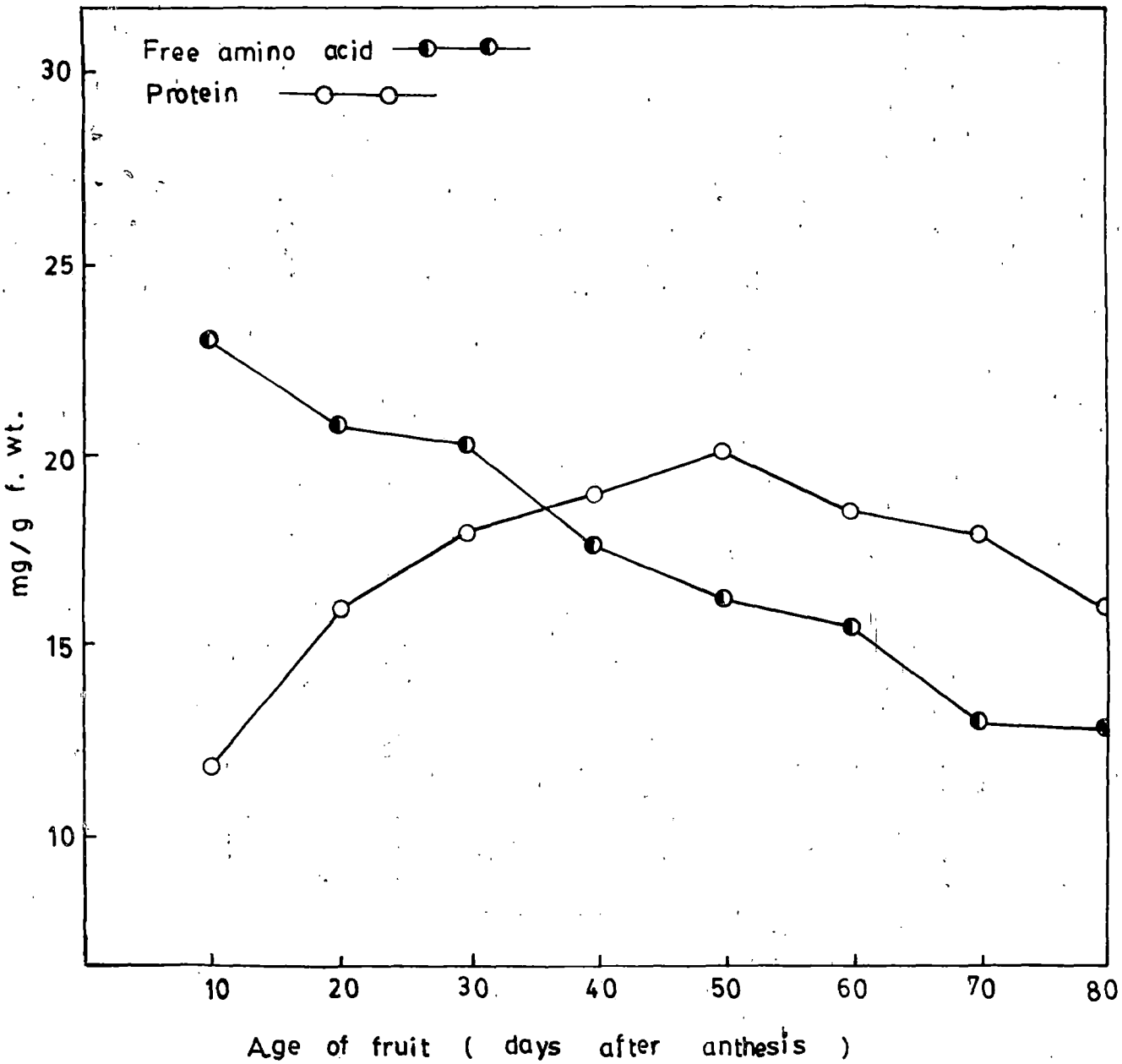

Fig. 21 : Free amino acid and protein content in developing fruit of S.vigrum Dunal.

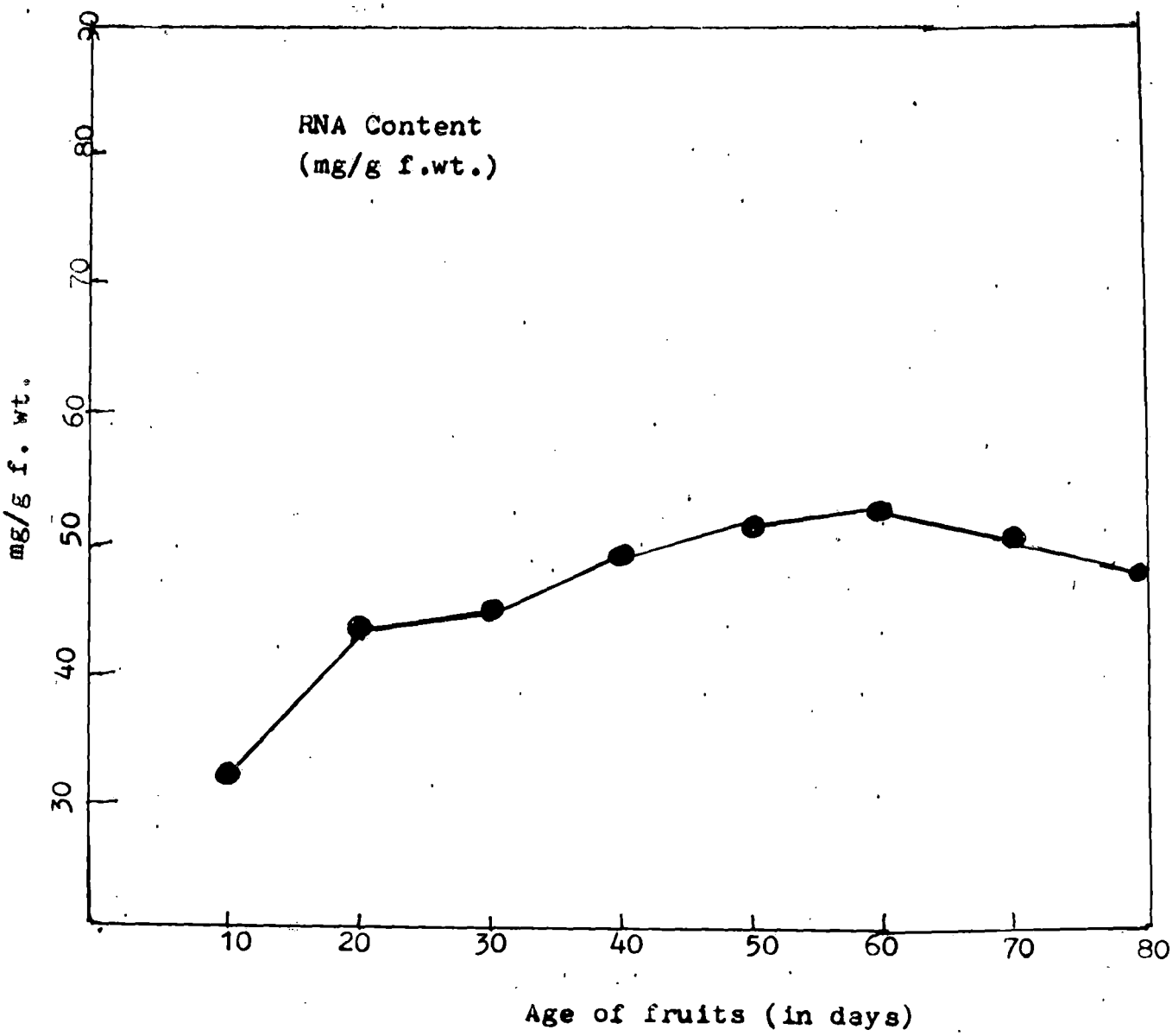

Fig. 22 : RNA content in developing fruit of Solanum viarum Dunal.

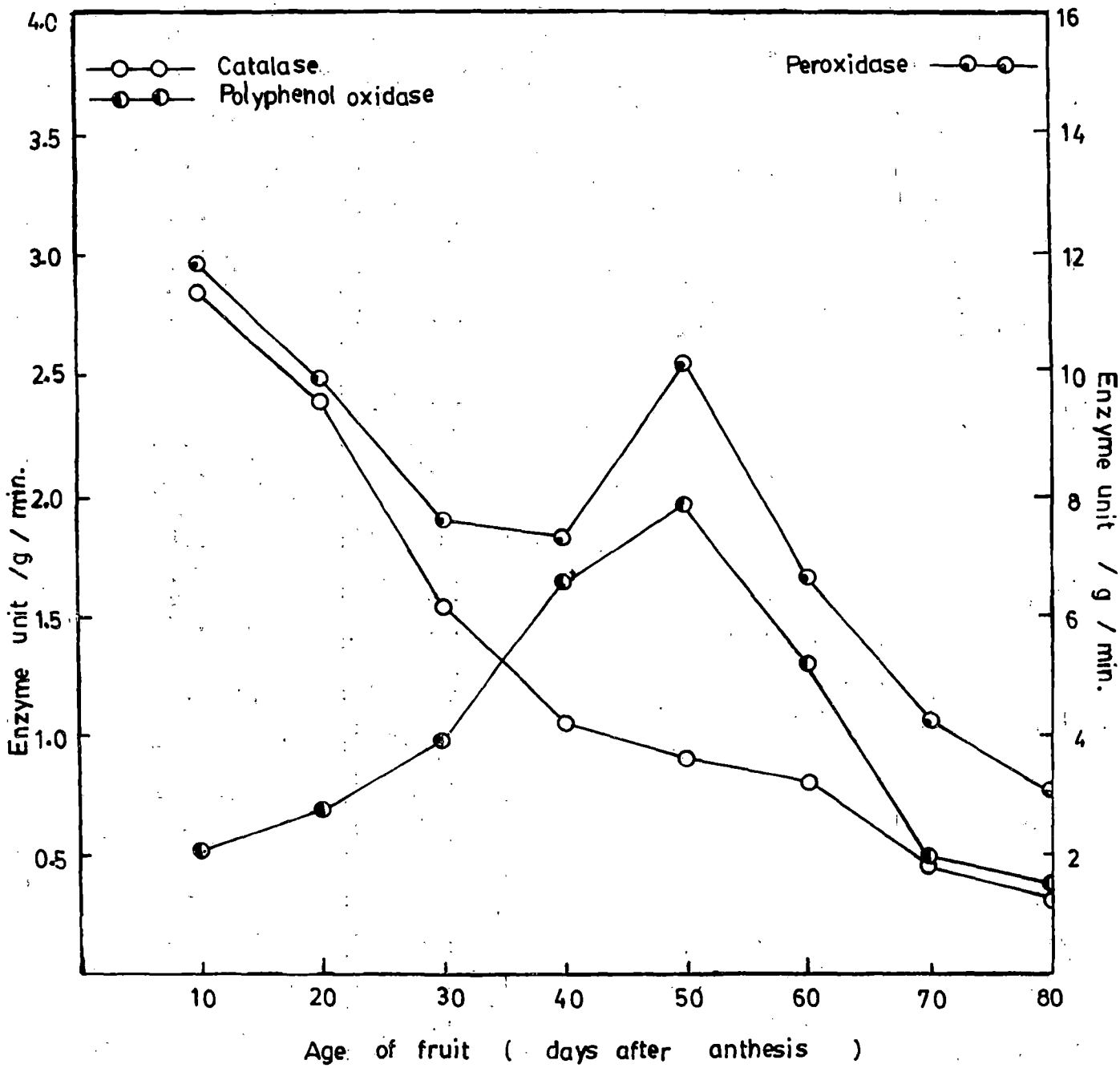

Fig. 23 : Activity of Peroxidase, Catalase and Polyphenol oxidase in developing fruits of Solanum viarum Dunal.

RESULT AND DISCUSSION

Fruit development is usually considered to start after anthesis, but early aspects of fruit development are initiated soon after flower induction (Noggle and Fritz, 1977). Fruit growth after anthesis has been studied for many plants. Many fruits have growth patterns of the simple sigmoid type common to cells, tissues and organisms, starting with an exponential increase in size and then slowing in a sigmoid fashion. A second group of fruits has a more complicated growth curve, involving two periods of growth increases with a period of slow or suspended growth in between. The double growth curve is common to probably all the stony fruits.

While working on the survey of wild population of S. viarum Dunal in the environmental condition of North Bengal it has been noted that growth and development of fruit of the plant was maximum in June/July (rainy season) as compared to those occur in Dec/January (winter season). The mature fruit during rainy season has been observed to contain maximum number of mature seeds as compared to minimum number of seed in fruits of winter months. Moreover in winter abortive fruits have been noted in huge quantity and which

does have any mature seed within the large cavity of fruit.

The variation in the development of fruit at different seasons in a year has been observed by Gupta (1983). In connection with the effect of time of sowing on the growth and yield of S. viarum Dunal. (Gupta, 1983) observed that the fresh weight of total fruit per plant (1.92 kg) was noted (hervest time, rainy season) to be the highest in October sowing plants in contrast with these (0.45 kg) of plant sown in February/March (hervest time , winter). Table-28 shows that the fruits of S. viarum Dunal collected in the month of July (rainy season) are superior in various characters such as diameter, volume, fresh weight, dry weight, total number of seed and glycoalkaloid content per fruit to those characters of fruit, collected in January (winter). Kaul and Atal (1978) reported from Jammu that S. viarum Dunal required moderate climate with average day temperature around 30°C during its growth period. They specially mentioned that S. viarum Dunal is a long day plant and it fails to thrive well under extreame climate conditions and that growth and development is the best under wild conditions of climate where maximum and minimum temparature are about 35°C and 20°C respectively. From the fig.15, it appears that development of fruit during rainy season as well as during winter months follow the sigmoids type of

curve but the development of seed within the fruit during winter season showed certain abnormality. During the period, number of seeds per fruit decreased considerably though the fruit developed to the scenescent conditions but having with much less volume of fruit as comparable to those of rainy season. In certain cases seeds do not attain maturity and fruit contained much enlarged cavity surrounding placenta bearing a few abortive seeds.

It is unnecessary to point out that the fruits which contain large amounts of food materials must import them from other part of the plant (Leopold and Kriedemann, 1983). Mobilisation can permit fruit growth at the expense of materials in the leaves.

Growth of a fruit may be an expression of a wide variety of events from the development of air spaces leading into the fruit with mature seeds accompanied by increase in volume, cell division to cell enlargement associated with various types of tissue differentiation. Finally there may be preferential growth of any of several or successive morphological parts of fruit.

Table-28 shows the difference in number of seeds alongwith other parameters in different fruits collected in

Table - 28

Different parameters of fruit harvested during rainy & winter season.

No. of observation	Month of collection/ season	Diameter of the fruit (cm)	Volume of fruit (c.c)	Fresh wt. of fruit (gm)	Dry wt. of fruit (g)	Total No. of mature seed	% of glyco-alkaloid on dry weight basis
1	July (Rainy)	3.20	9.6	8.75	1.82	484	4.3
2	"	2.85	7.2	5.15	1.15	470	4.1
3	"	2.50	5.5	3.50	0.89	395	4.0
4	"	2.45	3.3	2.82	0.52	360	3.8
5	"	2.20	1.7	1.25	0.45	288	3.2
6	Jan (winter)	1.85	5.6	3.25	0.92	325	2.95
7	"	1.5	3.4	2.75	0.51	196	2.8
8	"	1.3	1.8	1.53	0.42	65	2.2
9	"	1.0	1.2	0.92	0.98	22	1.2
10	"	0.45	1.0	.82	0.08	-	-

rainy and winter season in North Bengal.

There is a puzzling inconsistency about the evidence for the role of seeds in fruit growth (Leopold and Kriedmann, 1983). Abundant evidence indicates that seeds regulate many aspects of fruit growth. Gupta (1983) showed positive correlation of number of seed with the diameter of volume of fruit and fruit weight of S. viarum Dunal. This observation holds good for the fruit growing in the rainy season but during winter months how the fruit having minimum number of mature seeds or even with small number of abortive seeds can grow in a manner vary similar to those in rainy season. Nitsch (1950) provided some evidence that stimulate fruit growth by providing auxins. The photosynthetic activity of pericarp of S. viarum Dunal may have some influence on the growth of the fruit but does no influence the growth of seed within it. Low temparature during winter months might have interferred the mobilisation of metabolites from other part of shoot to growing seed within the fruit. This is well reflected in the developing fruit with much abortive seed in winter (table-28). Swanson and Whitney (1953) showed that mobilisation of various metabolites of leaf has highly sensitive to temparature and low temparature reduces.

According to Swanson and Bohning (1951) and Hewitt & Curtis (1948) observed that optimum temparature for

translocation reaches between 20° and 30°C. Cold environment specially low temparature reduces the mobilisation of raw materials from leaf (Corlson *et al.*, 1972).

Fig. - 16 shows the production of glycoalkaloid content in the developing fruit of the plant sown during winter (October). The percentage of total glycoalkaloid content has been observed to increase in the fruit developed after 10 days from anthesis. The total glycoalkaloid content reached maximum of 3.9% when the age of the fruit was 60 days and the colour of the fruit was observed to be yellowish green. The value decreased to 2.15% after 80 days of developing fruit. Thus the biosynthesis of glycoalkaloid in fruit of *S. viarum* Dunal is dependend on differentiation and development of fruit.

Much of the work has been done on the biosynthesis of the spirosolone alkaloids such as solasodine and its glycosides. It is intimately associated with the biosynthesis of spirostane steroids having the same ring structure. The insertion of nitrogen into alkaloid occurs quite late in the biosynthetic pathway.

Acetyl CoA is generally accepted as the starting point for steroid biosynthesis. It is converted to cholesterol

via the mevalonic acid pathway. A series of hypothetical reactions has been suggested by Kaneko et al., (1975). According to him teinimine has been suggested to be the key intermediate which can be cyclized in a number of ways to produce solasodine and other related alkaloids. Experimental evidence for this hypothetical pathway, consists almost entirely in demonstrations that lebeled precursors can be converted to the anticipated final products (Roddick, 1974 ; Schreiber, 1974). The ring of carbons of the steroids structure and solasodine ought to be derived from either the methyl or the carboxyl carbons of acetic acid. Careful degradation after administration of radioactivity labeled acetic acid or mevalonic acid to S. laciniatum plants have confirmed the localization of the radioactivity in the anticipated carbons of solasodine (Guseva and Paseshnichenko, 1962 ; Guseva et al., 1961).

If the broad outline of aglycone synthesis can be discerned, the route for glycoside attachment is shrouded in mystery. Labeled aglycone was converted to solamargine and solasonine when administered to stem sections of S. laciniatum (Liljegren, 1971). An enzyme preparation was obtained from the leaves that could be converted to solasodine + UDP - glucose to glucoside of solasodine. Presumably there also exists an enzyme to form galactosidyl solasodine.

Solasodine aglycone is too insoluble, however that it is difficult to understand why glycosidation with increased solubility should await this late step in synthesis. Perhaps the glyco-sidating enzymes are relatively non specific and would be capable of using steroid intermediates on the level of cycloartenol or cholesterol. Alternately the demonstrated enzymes may serve as a scavenging role to prevent undesirable intracellular precipitation of any solasodine that might be formed from glycosidase attack on the glycoalkaloids.

The term secondary products have been used to mean that these compounds are derivatives of primary products. One of the outstanding characteristics of secondary metabolism is it's dependence on the developmental stages of the organ. In higher plants, formation of secondary products is expressed as a specific features of certain organs or tissues during restricted periods of their development and specialisation.

This phase dependence was shown in several instances to be due to the phase dependent formation of enzymes, synthesising secondary products, demonstrating that expressions of secondary metabolism is the result of a differentiation process (Luckner, 1971 ; Luckner et al., 1977) and this has been reflected during the estimation of maximum amount

of glycoalkaloid content (3.9% on dry wt. basis) after development and differentiation of the fruit at the age of 60 days after anthesis when the colour of the fruit changes from green to yellow (fig. - 16).

It has been noted that special stage of development of fruit in S. viarum Dunal is necessary. The amount generally reported to be maximum in fully grown matures berries (Bakshi and Hamid, 1971 ; Chandra et al., 1970 ; Choudhury and Hazarika, 1966). As the fruit ripens solasodine decreases (Kaul, 1976 ; Patil, 1967). Khanna and Murty (1972) did not find any decrease in alkaloid concentration as fruit ripens. The observation on the growth and glycoalkaloid content of S. viarum Dunal have been described by Saini et al., (1965). They have shown that the fruits accumulated maximum amount of glycoalkaloid after about 50-55 days of their development. Correll et al., (1955) have also reported the maximum concentration of solasodine at this age . Contrary to this, Sharma et al., (1979) have reported the maximum level of solasodine content in the over ripening berries of 80 days old. In the present investigation the solasodine content has been observed to decreased rapidly in over ripened berries.

Dynamics of solasodine biosynthesis and its accumulation have been studied by Varghese et al., (1979). They have indicated that the leaves and berry wall are the sites of biosynthesis of solasodine which then accumulates in the fruit pulp where it is maximum in amount. According to them solasodine content was maximum in the wall of the young berries with a faster decline in mature fruit. Exactly a reverse trend was observed in case of the fruit pulp where solasodine content increased with increasing age of the berries and reached to its maximum in the pale yellow berries, there after it rapidly decreased in over mature fruit (golden yellow). They observed that solasodine content of fruit pulp had positive correlation (+ 0.97) which appeared negative (- 0.57) in case of berry wall. They also pointed out that the contribution of the berry wall to the total effective material was much less.

But from the table-29, it appears that no trace of alkaloid was present in the berry wall accompanied by the fleshy part of the fruit other than the mature seed and mucilage. The mature seed contained 1.2% of glycoalkaloid (table-29). Though 25.0% of glycoalkaloid (table-29) on dry weight basis was observed in the mucilage surrounding seed in the mature fruit. Saini (1966) pointed out that glycoalkaloid was localised in the gelatineous layer covering the

Table - 29

Dry wt., % of moisture and glycoalkaloid content in different parts of mature fruit.

Parts of fruits	Fresh wt.(g)	Dry wt.(g)	% of moisture	% of glyco-alkaloid on dry wt. basis
Mucilage	100	3.85	96.15	25.0
Seed	100	10.15	89.85	1.2
Pulp (Rest of fruit other than seed and mucilage)	100	9	91	0

seed surface in the fruit. Bakshi and Hamid (1971) determined the solasodine content in the seeds and mucilagenous mesocarp separately, and reported about 4% solasodine in the mesocarp which was much higher than the seed (2%).

Varghese et al., (1979) mentioned only fruit pulp and fruit wall in connection with understanding biosynthetic site of glycoalkaloid in fruit. It is probable that they include seed and surrounding mucilage in the fruit pulp and did not consider the seed mucilage and rest of the fruit separately. As the fruit pulp included seed and mucilage it is obvious that the fruit pulp showed maximum amount of glycoalkaloid. But here in this chapter an attempt has been made to study the anatomical preparation of the seed with a view to understanding the morphological nature of the mucilage. Besides the anatomical preparation of pericarp has also been made to understand the morphological relationship of the cell layer to the individual part of the fruit.

The pericarp (fig. 17b & 17c) consists of an epicarp followed by a fleshy mesocarp and an endocarp which can hardly be distinguished in the vicinity of the seed coat. Cells of the outer epidermis are polygonal with more or less straight anticlinal wall. The outer tangential and lateral

walls are strongly thickened while the inner tangential walls are thin. The mesocarp is formed of 2-4 outer layers of cells, cellulosically thickened at the corners ; thickening being more in outer layer.

There is a layer of thin walled rectangular cells outside the testa. The layer is represented here as extra testa. The cell has length, 215-225 μ and breadth 55-70 μ. This layer has been observed to be ruptured in seed of 60 days old berry (fig. 18c). The testa is made up of cells which appear almost square or radially elongated in L.S., showing striated thickening on the inner tangential walls and inner part of the radial walls. While, the outer tangential walls are thin with some cellose projections. The radial wall of the thickened cells of the testa has been observed to become 60-70 μ. Next to thickened cells of testa there is a layer of obliterated parenchyma having thickness of 28-35 μ. This is followed by the layers of endosperm cells which are round in shape having 35-70 μ which again is followed by the layer of embryonic cells (fig. 18a,b & c).

This observation is very much confirmity with observation made by Corner (1976) with the exception that he did not mention the extra layer of parenchyma outside the thickened layer of testa in Solanum species. The presence of

extra testal layer of parenchyma may be considered as the first time to report in connection with understanding of the mature seed coat in Solanum.

From the anatomical preparation of developing seed the mucilage has been noted to originate from its outer parenchymatous layer of testa of the seed of S. viarum Dunal (fig. 18c). With this background it may be mentioned that the seed with the fruit should get prominance so far as the site of synthesis and accumulation of glycoalkaloid are concerned. Out of all other parts in the fruit, seed of S. viarum Dunal may be considered as the main site of biosynthesis of glycoalkaloid for several reasons ; i) The seeds contain a considerable amount of glycoalkaloid as compared to the pericarp which did not show any trace of alkaloid. ii) Carpesterol is biosynthetically related to solasodine. It is expected that at the site of synthesis solasodine carpesterol should be present. But pericarp did not show any trace of carpesterol and glycoalkaloid. On the other hand carpesterol is present basically in the thickened radial wall of the testa. Thus it is expected that solasodine glycoalkaloid synthesis occurs within the developing seed and not in the pericarp. Carpesterol which is biosynthetically related to solasodine was obtained solely from the testa of the seed (table-11) having characteristic thickness of the

wall and not in any other parts of fruit. iii) In winter the berry developed considerably with less number of mature seeds per fruits (mostly abortive) accompained by less amount of glycoalkaloid content (table-28). Fruits with much volume but having no mature seeds did not show any production of alkaloid (table-28). During winter, fruit contained watery mucilage in the fruit as compared to thick gummy mucilage during rainy season. It is expected that seed is something to do with the biosynthesis of glycoalkaloid. Further investigation is necessary to understand the problem after studing with labelled precursors.

Fig-19 shows that soluble and insoluble carbohydrate content are maximum in amount immediately at the begining of the development of the fruit. They gradually declines in the fruit at the age of 20 days. After that they gradually increase upto 50 days development of fruit and again declines upto 80 days. It is expected that much carbohydrate of both the forms are utilized due to high metabolic activity at the begining of fruit formation. On the other hand gradual increase of total nitrogen was observed from the begining of fruit development upto 50 days when it showed maximum in amount. This has also been observed to decline after 50 days of development of fruit to its senescent condition (fig. 20). The accumulation in the protein in the fruit also followed

the same trend as observed in connection with the total nitrogen (fig.-21). On the other hand gradual decline of free amino acid has been observed during the development and maturity of the fruit (fig.-21). It is probably due to utilisation of amino acids for the synthesis of enzyme protein required for high differentiation of fruit. This is supported by the synthesis of RNA at highly differentiated stage of the fruit. RNA content has been observed to increase upto 60 days of development of fruit (fig.-22).

Protein content fall rapidly as senescence develops. The changes of colouration of fruit from green to yellow is well marked when the fruit attains 50-60 days of its development. At that time the glycoalkaloid content have been observed to be maximum (fig.-16). It is possible that the decline of protein may be structurally associated with deterioration of the chloroplast (Woolhouse, 1967). It is observed in case of detached leaves that chlorophyll and protein content fall rapidly with approaching senescence (Lewington et al., 1967 ; Vickery et al., 1937). Pigment changes during fruit ripening also ordinarily involve decline in chlorophyll and transformation of carotenoids (Chichester and Makayama, 1965 ; Puech et al., 1976). The decline of chlorophyll, skin protein and pulp protein have also been observed to decline during senescent of fruit (Tosh et al.,

1977). Though the behaviour of RNA has not been observed in developing fruit, the deterioration of synthetic activity during leaf ageing leads one to expect a decline in RNA ; infact such a decline appears to be general (Bottger and Wollgiehn, 1958). Abrams and Pratt (1968) have observed that in leaf disc the progress of senescence may be associated with a decline in RNA. Wright et al., (1973) have found that some species of t-RNA are lost during senescence of leaves and cotyledones. Thus increase of protein and RNA at the developing stage upto 60 days is probably due to the anabolic process involved during its differentiation.

Steroidal alkaloids are a typical "alkalodia imperfecta" (Roddick, 1974) because the nitrogen is added very late in their biogenesis in contrast to ordinary alkaloids derived in most cases from amino acids.

Out of total nitrogen (fig.-20) and carbohydrate (fig.-19) content in the developing fruit, the carbohydrate and alkaloid have been observed to show the same trend of enhancement in their total glycoalkaloid content sequencing with the maturity of the fruits. This confirm to the typical steroidal structure of the alkaloid, solasodine which is a product of "high carbohydrates" rather than "high nitrogen" condition (Nowacki et al., 1975). The location of the

glycoalkaloid, being the extra cellular mucilage may protect against the loss of solasodine that commonly occur in other species during fruit ripening. Since in most other species the alkaloid is intracellular (Mann, 1978).

Peroxidase has been recognised as a principal enzyme controlling secondary metabolism. Different forms of peroxidase has been found in the protoplast and in the periplasmic space of higher plant (Mader et al., 1975 ; Mader, 1976). They have also been noted to be present in the endoplasmic reticulum, dictyosome, tonoplast, vacuoles, plasma membrane and chloroplast (Liu and Lamport, 1974 ; Parish, 1975 ; Henry, 1975a ; Mader et al., 1975). They are very much involved in phenolic biosynthesis (Nakamura et al., 1974). Fig.-23 shows that in the developing fruit that peroxidase activity has been observed to be maximum at the onset of development of fruit. It gradually declines upto 40 days of development of the fruit. It becomes maximum when the fruit attains the maturity of 50 days of development. After that it has been observed to decline upto 80 days of development. As the fruit contains the high content of phenolic compounds in the fruit there is a possibility of its involvement in phenolic biosynthesis, as the total phenol has been observed to be maximum during the same period of development of fruit. Moreover, peroxidase has been used in

a model system for the conversion of methionine (Yang, 1967) or peptides of methionine into ethylene (Ku and Leopold, 1970). Other systems containing peroxidase are also capable of generating ethylene from various substrates (Kumamoto et al., 1969 ; Takeo and Lieberman, 1969). Ethylene is universally accepted to be involved in the fruit ripening; the peroxidase activity in fruit of Solanum viarum Dunal may therefore have some role on the ripening of fruit.

The informations about the activity of catalase in the developing fruit is lacking however, a number of controvertial results have been published during detached leaf senescence. Both increased and decreased level of catalase activity have been reported. For example, catalase activity decreased in tobacco (Farkas et al., 1963 ; Parish, 1968) and increased in wheat and barley (Farkas et al., 1963) during detached leaf senescence. Kar and Mishra (1976) also noted that this enzyme decreased during senescence of both attached and detached leaves of rice. Thus according to Kar and Mishra (1976) the trend in change of enzyme activity during senesence is species specific. Its activity increased during senesence in some plants and decreases others. However, During the present investigation it have been noted to decrease according to maturity of fruit in Solanum viarum Dunal (fig.-23).

As the phenolics are the substrates for peroxidase and polyphenol oxidase enzymes an attempts has been made to determine the level of total phenolics during the senescence of fruit. The (fig.-20) shows gradual increase of total phenol upto 60 days and than decreases. This is very much co-related with the enzymes activity of polyphenol oxidase as represented in fig. (23).

Phenolic synthesis is a characteristic and universal feature of plant metabolism. It is now widely accepted by plant biochemist that phenolics are not end products that accumulate unchanged in plant cells, instead, they are thought to be part of dynamic equillibrium, even produced in quantity, there is continual synthesis, turnover and degradation (Harbone, 1980). The fate of simpler phenolic acids in plant tissues has been extensively studied (Ellis, 1974), one main reaction is the oxidation of hydroxy cinnamic acids to hydroxy benzoic acids. In general catechol derivatives are formed and these finally undergo ring cleavage to aliphatic acids and then to CO_2. Most of the phenolics, specially the caffeic acids and benzoic acids, may effect plant growth by interaction with one or other of the major classes of plant hormones, such as the auxins (Stenlid, 1968 and 1976 a). They also affect biosynthesis of ethylene which is important for fruit ripening. Generally they serve as necessary

co-factors for ethylene biosynthesis from methionine (Mapson, 1970). They also serve as co-factors to peroxidase like enzyme. The relatively recent discovery is that certain phenolics, particularly caffeic acid, occur in plant chloroplasts (Saunders and Me clure, 1976) and affect photosynthesis. It has already been mentioned (table-11, chapter-II) that, high accumulation of carpesterol has been observed specially at the yellow stage of fruit. Carpesterol is unique in its structure that each molecule contains, one molecule of benzoic acid (Beister and Sato, 1971 ; Goad, 1967 ; Schreiber, 1968 ; Schutte, 1969). Due to synthesis of a considerable amount of benzoic acid, during development of fruit, the phenolic content is expected to become high. Increased content of phenol may appear to play role as fungicidal and bacteriocidal agent. Moreover, the phenolic constituents especially the benzoic acid in S. viarum may serve as an allelochemic constituent for maintance of some insect like Spilotethus,(Basu & Laha, 1982). Thus, the fruit of Solanum viarum Dunal has a great role in connection with plant-animal relationship.

During the later stages of development of fruit metabolic processes are initiated and which characterize fruit maturation. At about the time, when the fruit reaches its maximum size, the fruit tissue undergoes striking changes

in structural and metabolic activities, a major feature of which is a burst in respiratory activity. This burst in fruit respiration is known as climacteric (Noggle and Fritz, 1977). The increase in rate of respiration is short lived and subsidies as the fruit ripens and enters the senescence phase of development. During the increase in respiratory activity of climacteric process the carbohydrate liberated during the conversions of glycoalkaloid to aglycone may be helpfull to serve as respiratory substrate. This may be correlated with the decrease in glycoalkaloid content during the scenescent phase of the fruit (fig. 16). Moreover, the protein and other degraded products at the senescence of fruit, may also be utilised during the climacteric process and for which further investigation is needed.

S U M M A R Y

Fruits of S. viarum Dunal collected in the month of July (rainy season) are superior in various characters such as diameter, volume, fresh wt., dry wt., total no. of seed and glycoalkaloid content per fruit to those of fruits collected in January (winter).

Growth of both types of fruit showed typical sigmoid curve.

The same treand in increase of glycoalkaloid content, soluble and insoluble carbohydrate has been observed in between 20-80 days of development of fruit.

Glycoalkaloid content was observed to be maximum in the fruit having the age of 60 days when the colour of the fruit changed from green to yellow, then it declined. Gradual increase of total phenol was observed upto 60 days of development of fruit, after that it declines.

Total N, Protein and RNA showed the same treand of accumulation in the developing fruit.

Free amino acid was observed to be maximum in the fruit, developed 10 days after anthesis, then it gradually declined upto 80 days of development of fruit.

Peroxidase activity was observed to be maximum at the initial stage of the development of fruit upto 40 days then gradually increase to it maximum limit when the fruit reached its development upto 50 days. After that it declined. On the other hand polyphenol oxidase activity was minimum at the initial stage of development of fruit and increased upto 50 days to its maximum limit then it decline. The catalase activity was observed to be maximum at the initial stage of development of fruit. Then it gradually decline upto 80 days.

In anatomical preparation, a layer of extra testal parenchyma was observed outside the hard coat of testa. The cells parenchyma ruptured after 60 days of development of fruit. This layer has been pointed out for the first time to be related to the formation of mucilage.

Glycoalkaloid was estimated in different parts of fruit, such as mucilage, seed and pulp covering rest of fruit other than seed and mucilage. The mucilage and seed showed 25% and 1.2% respectively of glycoalkaloid on dry wt. basis. The pulp did not show any trace of glycoalkaloid.