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A New Method for Quantitative Determination of Solasonine from Developing Berries of *Solanum khasianum* Clarke

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An easy quantitative estimation of solasonine was established colorimetrically in presence of resorsinol solution having concentration ranging from 10^{-6} ppm to 10^{-1} ppm obeying Beer's law. This method can be utilized for the determination of glycoalkaloid from very small amount of dry powder. Solasonine content was determined at different stages of fruit development. Maximum solasonine content was found after 80 days of fertilization. The correlation of solasonine content with the diameter and dry weight of the fruit is discussed.

Solanum khasianum Clarke is well known for the high glycoalkaloid content of its fruits. The glycoalkaloids extracted from fruits were mostly solasonine which on acid hydrolysis gave solasodine along with one mole each of rhamnose, glucose and galactose[1,2]. Solasodine (Δ^5,22β,25α-spirosolen-3β-01,$C_{27}H_{43}O_2N$, mol. wt. 413.62, m.p.-200-202°C) can be converted into the key intermediate 3β-acylΔ^5, 16 Pregnadine 20-one, from which almost all the corticosteroid preparations including sex hormones can be synthesised. This glycoalkaloid was originally isolated and identified from *S. sodomeum* Linn[3]. Various other names were given to similiar compounds until these were grouped together under the name solasonine by Sato *et al.*[4,5]. Different procedures were adopted by different authors[6-13] for isolation, purification and estimation of the glycoalkaloid. present investigation deals with the establishment of a new method for quantitative determination of solasonine and observation on changes of solasonine content at different stages of developing berries in *S. khasianum* Clarke.

Fresh berries were collected locally during June-July, 1979 and oven dried at 52°C. Dried berries were powdered and were soxhlated with petroleum ether (40°-60°C) for 12 hr; the residual meal was refluxed with sufficient quantity of methanol for 3 hr; the methanolic extract was filtered and concentrated. The concentrate was mixed with acetic acid (3%) and heated to remove the last traces of methanol. The acid solution was then made alkaline with ammonia in hot condition when the glycoalkaloid separated out. The crude glycoalkaloid was purified into different fractions by coloumn chromatography on aluminium oxides. One of the fractions obtained from methanol was identified as solasonine by authentic marker through TLC on Silica gel G using butanol:acetic acid:water (10:3:8) and (4:1:5) and chloroform:acetone (99:1), the R_f being 0.55, 0.65 and 0 respectively; m.p. 284°-286° (decompose), sulphuric acid in cold gave colouration of an amber red, and with concentrated nitric acid a brownish yellow colour was observed. Glycoalkaloid was also identified with UV and IR spectrum analysis.

Estimation of the percentage of Solasonine was carried out at the five developmental stages of berries of *S. khasianum* Clarke. The developmental stages were green, yellowish green, light yellow, deep yellow and light brown (senescence). Berries of each stage ranging from 10 days to 140 days after fertilization were collected from 20 different plants. They were randomly mixed, oven dried (52°C) and powdered for estimation of solasonine. 5 mg of dried powder was refluxed in hot methanol. 1 ml of the methanolic extract was subjected to TLC on silica gel G for purification of solasonine. The eluted solasonine was

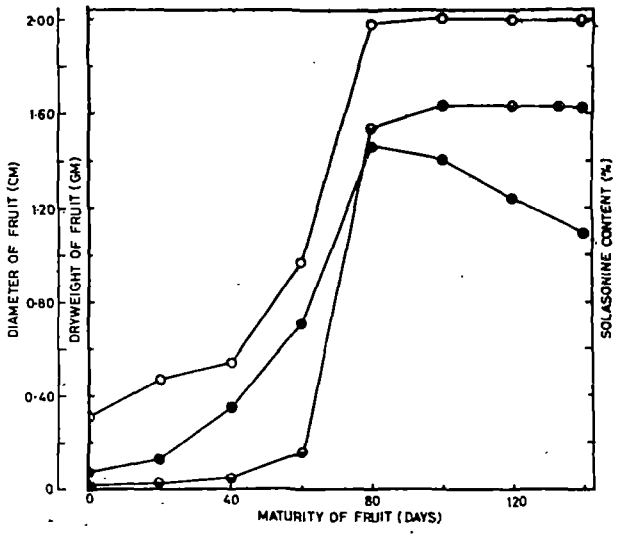

Fig. 1—Changes of diameter, dry weight and solasonine content in developing berries of *S. Khasianum* [Diam of the fruit (cm) O—O. Dryweight of the fruit (g) ◒—◒. Solasonine content (%) ●—●]

taken in 2 ml methanol (hot) from which only 0.5 ml methanolic solution was mixed with 1 ml acetic resorcinol and 1 ml sulphuric acid for the development of orange red colouration having absorbtion maxima at 510 nm. The colour was stable after 5 min. percentage of solasonine was calculated from the prepared standard curve ranging from 10^{-6} ppm to 10^{-1} ppm solution of solasonine which was observed to obey Beer's law. The diameter(cm) and dry weight (g) of berries were also recorded during the developmental stages (Fig.1).

The procedure for the quantitative determination of solasonine is much more easier than the earlier ones[6-13]. No method utilising acetic resorcinol and sulphuric acid has yet been observed in connection with the quantitative determination of solasonine. This is advantageous because of the fact that glycoalkaloid can be determined from low concentration of solution as 10^{-6} ppm which was not possible earlier. Again, with the help of this method only 5 mg of dry powder was found to be sufficient for the estimation of solasonine. Nobody has taken such a very small amount of dry powder into consideration during the estimation of glycoalkaloid. This method is also less time consuming as total duration for the estimation is supposed to cover only 40 min and following TLC purified solasonine can be obtained. Estimation of solasonine from different developmental stages of berries (Fig.1) indicates that solasonine content increased with the maturity of berry and the maximum percentage of solasonine was recorded when the colour of berries were yellowish green (80 days after fertilization of the flower). This observation was similar to other reports[14-17], though other workers stressed the maximum yield of solasonine in purely yellow stage[18]. The size of the berry (cm) and dry wt (g) of fruit were shown to have similar trend with solasonine content upto 80 days of age of the berry, afterwards these maintain almost the same level, but solasonine content decreased rapidly (Fig.1).

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Table 1. The mean, minimum and maximum values for various component characters in *S. khasianum.*

Characters values	Dia./fruit (cm)	No. of seeds/fruit	Fresh wt. fruit (g)	Yield/fruit (g)
Minimum	1.53	42	1.600	0.460
Maximum	2.51	268	6.270	2.077
Mean	2.091	147.85	4.497	0.975

indirect effect of component characters of single fruit in *S. khasianum* with the yield instead of taking various characters of the plant.

MATERIALS AND METHODS

The material consisted of 2000 fruits collected from the experimental plot (300 sq m) of North Bengal University campus in May 1979. The plants were grown in a randomized block design with three replications. Each plot consisted of four rows of five plants with the spacing of 100 X 100 cm between plants and rows. Observations were recorded on various component of a single fruit i.e. diameter, number of seed, fresh wt and dry wt (yield). Diameter was measured with the help of a slide calipers and each value represented the mean of four readings. The total number of seeds counted from each fruit separately were recorded. Correlation coefficients, regression coefficients and regression equations were based on the method of Panse & Sukhatme (1978). Path coefficient analysis was carried out following the procedure of Dewey & Lu (1959).

RESULTS AND DISCUSSION

The mean, maximum and minimum values for all characters are presented in Table 1. Regression coefficient, regression equations with yield and its components are shown in Table 2. All the regression coefficients were found to be highly significant. From the regression values it emerges that with an unit increase in the diameter/fruit, number of seeds/fruit and fresh weight/fruit there would be an addition of 1.3070, 0.0056, 0.2656 to yield/fruit, respectively. Correlation coefficient with components of direct and indirect effects are presented in Table 3. The value + 0.802 was observed to be the highest when correlation was measured in between yield and number of seeds/fruit.

Table 2. Regression equation of yield and its components.

Character	Regression eq.	F value	
Dia./fruit & yield/fruit	—1.757+ 1.3070x	27.4798	**
No. of seeds/fruit & yield/fruit	0.1371+ 0.0056x	31.6618	**
Fr. wt./fruit & yield/fruit	—0.2189+ 0.2655x	32.8208	**

** Significant at 1% level.

Results (Table 3) also showed that the number of seeds/fruit had maximum direct effect on yield. The direct effect of fresh weight/fruit was found to be positive. The diameter/fruit showed negative and low direct

Table 3. Correlation coefficient with direct and indirect effect of yield and its attributes in *S. khasianum.*

Characters	Dia./fruit (cm)	No. of seeds/ fruit	Fr. wt./fruit (g)	Correlation with yield
Dia./fruit	*—0.003*	0.586** (0.947)	0.284** (0.992)	0.777** (0 777)
No. of seeds/fruit	—0.086	*0.619*	0.282** (0.954)	0.813** (0.802)
Fr. wt./fruit	—0.089	0.590	*0.296*	0.797** (0.796)

Correlation coefficient values within brackets; **significant at 1% level; values in italics denote their direct & residual effects=0.0812.

effect. Saini (1966) is of opinion that the mucilage layer surrounding each seed was very important for solasodine production. Saini & Biswas (1967) further enumerated that the outermost layer of developing seeds disintegrated to produce mucilage layer in the fruit, and in this part of work number of seeds/ fruit was also found to be important in connection with fruit yield. Thus it is suggested that the character like fresh weight/fruit should be considered as an important yield component but the major emphasis should be stressed on the number of seeds/fruit for better fruit yield in *S. khasianum.*

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INFLUENCE OF LIGHT AND SHADE ON *SOLANUM KHASIANUM* CLARKE

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Solanum khasianum clarke (Solanaceae) is well known for the high glyco-alkaloid content of its fruits. The glyco-alkaloid solasodine ($C_{27}H_{43}O_2N$; mol. wt. 413.62 m.p. 199-202°C) can be converted into the key intermediate 3β – acyl – 4^5, 16-pregnadine-20-one from which almost all the cortico-steroid preparations including sex hormones can be synthesised. Hooker (1883) first recorded this plant from Khasia hills. Sen Gupta (1961) described this plant in detail and Maiti *et al* (1964) reported the yield of 5.4% alkaloid in the fruit on dry weight basis.

In North Bengal this plant grows well under natural condition. Present investigation was an attempt to study the effect of natural light and shade on different morphological characters of *Solanum khasianum* clarke which are associated with the yield.

Seeds were sown in seed bed to raise seedlings which were later transplanted to earthenware pot (36 cm) containing garden soil and leaf manure (3 : 1). Twenty pots each having a single plant were kept under each treatment of light and shade. Observations were recorded on individual plant on the plant height (cm) spreading (cm), frequency of spine on leaves, days to first flower formation, days to first fruit formation, days to change of fruit colour from green to yellow, total number of fruits per plant, average size of the fruit (volume/fruit in c.c. equivalent to the amount of water it dispersed, number of seeds per fruit.

Height of the plant in both light and shade condition was same but spreading was more on light (Table 1). Frequency of spine on leaves have been found very less on shade condition but it was hard and more in light. From the Table 1, it has been observed that days to first flower and fruit formation was very short in light condition in comparison to shade plants. Days to change of fruit colour from green to yellow was more or less similar in both the conditions. In light condition, number of fruits per plant, fruit size and number of seeds per fruit was also found to be large than those of fruits grown in shade which were small in size, less in number and number of seeds per fruit was also less (Table 1).

TABLE 1

Mean performances of different morphological characters of Solanum khasianum clarke in light and shade condition

Treatmen	Height of the plant (cm)	Spreading (cm)	Average number of spine on upper surface of Leaf	Days to first flower formation	Days to first fruit formation	Days to change of fruit colour from green to yellow	Total number of fruits per plant	Fruit size (c.c.)	Number of seeds per fruit
Light	149	80	23.58	70.65	115.90	27.20	62	8.20	269.85
Shade	130	80	11.07	85.70	148.80	27.30	26.40	3.77	60.70

From the present investigation it appears that the habit of the shade plant is straggling whereas in light condition it was found to be erect. The days to flower and fruit formation is of short duration in light condition which is very much desirable for commercial cultivation of this species. So, viewing on this point, light condition is favourable than shade. Again, total number of fruits per plant increases in light condition than shade. So, yield of this plant would be better in light condition. Fruit size and number of seeds per fruit also shows similar trends in light condition. The only advantage of cultivation in shade condition is that the collection of fruits (harvesting) becomes easier due to formation of less spines on the leaves.

From the above consideration it may be concluded that the light condition is much more desirable than shade condition for the growth development and ultimate yield of fruits of this species.

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GROWTH PERFORMANCE OF *SOLANUM KHASIANUM* CLARKE IN NORTH BENGAL

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Yield is dependent on a number of component characters. The study on the association between any pair of characters in relation to yield of plant parts having economic importance is of immense value at the time of selection of superior genotypes. *Solanum khasianum* Clarke is well known for the high solasodine content of its fruits. Maiti et al. (1964) reported the maximum yield of solasodine (5.4%) in fruits on dry weight basis. Experimental cultivation of this plant has been succssfully achieved in southern part of West Bengal (Charaberty & Basu, 1973) Saini & Safui,1974). The study of the yield of fruit was observed previously in clayey soil (Chakraverty & Raychoudhuri, 1974) as well as in saline alkali soils (Kapoor & Misra, 1976). So, it has been felt highly desirable to cultivate this plant on large scale in the acid soils of North Bengal. The present investigation deals with the association of individual component with the yield of *S. khasianum*. Plants were grown in the cultivated plot of Phulbari (Dist. Jalpaiguri) in the year 1978. 50 different soil samples were analysed for the experiment. The soil contained 0.22% total N, 27.50kg/ha available P_2O_5 and 110/20kg/ha available K_2O with pH 4.2 and T.S.S.O. 80 mmhos/cm. The meteorological data observed during the experiment are given in Table 1. Correlation coefficient were studied following the procedure of Bailey (1965). Mean values for various characters, correlation coefficient between yield of fruits and its components and the relationship between the component themselves are presented in Table 2. Results revealed that all the correlation values with yield were positive. The negative correlation values were observed between spine length and various other characters like spread of leaf length and leaf breadth. Significantly correlated

Table 1. Meteorological data at Phulbari (Jalpaiguri Dist.) during the experimental period.

Month	Temperature Max°C	Temperature Min°C	Total rainfall (mm)	Humidity (%)
Feb.	26.40	13.40	12.95	60
Mar.	30.50	17.80	30.00	57
April	32.20	19.90	115.20	70
May	33.45	20.15	338.79	75
June	30.10	19.40	710.00	82
July	29.85	19.10	776.10	85
Aug.	29.80	17.80	662.80	79
Sept.	28.90	17.60	536.90	79

Table 2. Mean values and correlation coefficients of different characters in *S. khasianum*.

Characters	Plant height	Spread	No. branch	Leaf length	Leaf breadth	Spine length	Total No. fruits	Fruit fr. wt.	Yield of fruits
Mean value	55.2cm	36.7cm	15	7.8cm	7.2cm	1.0cm	12	39.5g	8.1g
Plant height		+0.16	+0.30	+0.18	+0.18	+0.28	+0.30	+0.30	+0.30
Spread			+0.13	+0.34	+0.47*	—0.15	+0.56	+2.77△	+1.94△
No. branch				+0.42*	+0.39*	+0.21	+0.23	+0.27	+0.25
Leaf length					+0.96△	—0.33	+0.38	+0.36	+0.21
Leaf breadth						—0.39	+0.34	+0.32	+0.06
Spine length							+0.13	+0.12	+0.10
No. fruits								+1.06△	+1.05△
Fruit fr. wt.									+0.99△

*Significant at 5% level; and △ at 1% level.

values have been represented in Table 2. From the result it emerges that the major emphasis should be strssed on the characters like spread, total number of fruits and fresh weight of fruits at the time of selection for better fruit yield of *S. khasianum* during the study on agronomy.

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Presence of high alkaloid content in the fused fruits of *Solanum khasianum* Clarke

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The fruits of *Solanum khasianum* Clarke is a rich source of solasodine, used as a precursor for the synthesis of cortisones and related products (Maity *et al.*, 1964). The alkaloid content has been reported from 0.5 to 7.4 percent on the basis of dry weight of normal fruits (Hamied, 1965; Saini, 1966). Occurrence of fused fruit (fig—1) is now being reported for the first time from the fertiliser treated plant applied as $N_{40}P_{40}K_{80}$ doses (calculating on kg/ha basis) in the experimental garden of North Bengal University. The age, stalk length, dry weight, number of seeds, percentage of alkaloid and nitrogen of the control and fused fruits are given in Table—1. Nitrogen and alkaloid have been estimated following the procedure mentioned earlier (Birner 1969; Vogel 1961; Gupta and Basu 1981).

Table—I

Comparative data of fused and control fruit in S. khasianum.

	Fused	Control
Age (in days)	197	220
Stalk length (cm)	0.6	0.5
Dryweight (g)	1.130	0.725
Number of seeds	284	142
Percentage of alkaloid	5.96	4.20
Percentage of nitrogen	0.14	0.18

Number of seeds per fruit and the percentage of glycoalkaloid per fruit in fused condition is greater than in those of the control.

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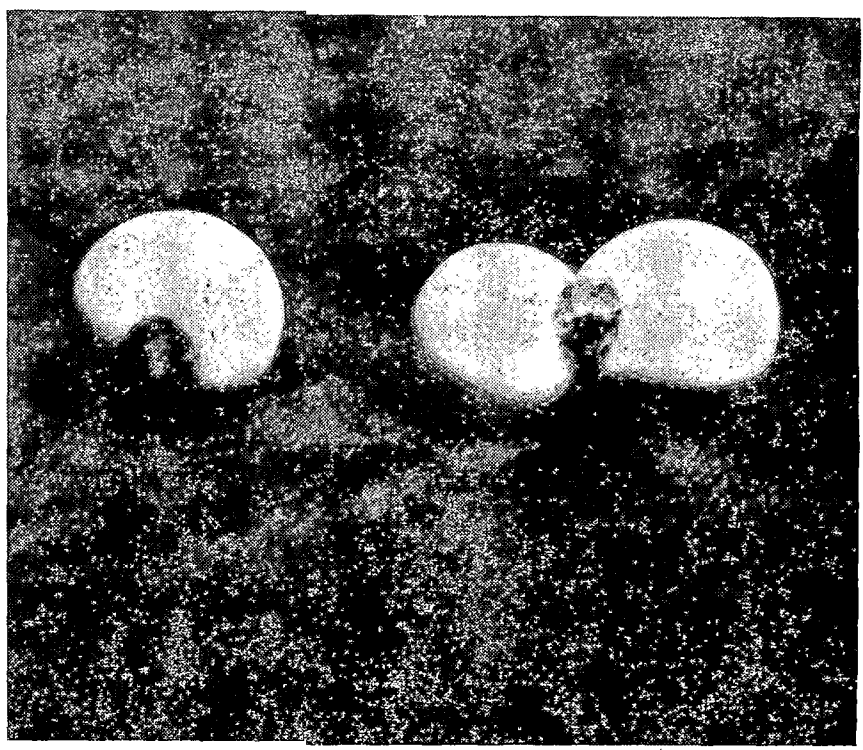

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Fig.—1 : Fused and control fruit of *S. khasianum.*