A B S T R A C T

Solanum viarum Dunal (family - Solanaceae) has recently come into prominance due to its high content of glycoalkaloid. The ripe berries of the plant contain various steroidal glycoalkaloids, the aglycone part of which is solasodine, a C_{27} steroidal alkaloid and a nitrogen analogue of diosgenin. This is generally utilised commercially as a basic precursor for the preparation of 16-Dehydropregnenalone acetate (16-DPA) from which the corticosteroids and contraceptives are being manufactured on commercial scale.

This plant has been noted to grow luxuriantly at the foot hills in the district of Darjeeling and Jalpaiguri of North Bengal ascending at an altitude of 7500 ft and it has enough potentiality of commercial utilisation to boost up the steroid industry in the region. It is obvious therefore that scientific investigation on this plants from utilisation point of view will be of much economic value.

The productivity of glycoalkaloid is very much dependent on growth and development of the plant. Thus plant physiological investigation has been carried out to solve. Certain problems which have so far been neglected.

About 4 percent of glycoalkaloid on dry weight

basis has been noted to be accumulated in the fruit of Solanum viarum Dunal. Previously much emphasis has been given on the fruit in connection with the biosynthesis and accumulation of glycoalkaloid. It is believed that accumulation of total glycoalkaloid is mainly retricted to the mucilagenous part of the fruit and to some extent to the fleshy pulp of the same disregarding the role of seed on biosynthesis and accumulation of glycoalkaloid. Moreover, the morphological nature of the mucilagenous layer has not worked out. Thus phytochemical analysis has been carried out giving much emphasis on separate parts of the fruits, i.e. seeds, mucilage and rest of the fruit. Moreover, chemical analysis has been performed taking different parts of the seed i.e., seed coat and cotyledonary embryo. In this respect this should be treated as the first time to investigate in connection with isolation and characterisation of chemical constituents in separated parts of the fruit.

Phytochemical analysis has been performed following conventional method of soxhleting air dried plant parts first with petroleum ether followed by methanol. Crude petroleum ether part has been column chromatographed over alumina and pure crystals have been isolated after elution with suitable solvent starting from non-polar to polar one and with their mixture. Methanolic extract has been separated into phenolic acid, acid, basic and neutral fractions following conventional method and pure crystals have been separated from the sub-fractions ether by column chromatography or by fractional crystalisation. Chemical constituents are identified after studying comparative behaviour in m.p., m.m.p., TLC, PC, spectrum of UV, IR, NMR and Mass of the isolated products with those of authentic samples.

Petroleum ether extract of the seed shows the presence of β-sitosterol, lanosterol and carpesterol, a unique phytosterol having biosynthetic relationship with solasodine. Phenolic acid fraction showed no appreciable amount of solid to be identified. Basic fraction contained a considerable amount of residue identified as Solasonine a glycoalkaloid, the aglycone part of which is solasodine. Acid fraction shows the presence of unidentified solid having 65-68°C. β-sitosterol has been identified from the neutral fraction. Ether insoluble methanolic extract shows the presence of 0.9% of glycoalkaloid on dry weight basis, the component of which has been identified to be solasonine and solamargine. Solasodine has been identified as the aglycone part of both the alkaloids. The presence of glycoalkaloid in seed may be treated as the first time to report in the plant. While working on the phytochemical analysis of different parts of the seed i.e., seed coat and cotyledonary embryo, the former shows the presence of high content of carpesterol. The rest of the seed other than seed coat shows the presence of β-sistosterol and lanosterol and 0.9% of glycoalkaloid on dry weight basis.

The petroleum ether fraction of mucilage does not show the presence of any appreciable amount of crystals, though methanolic part shows the presence of high content of glycoalkaloid and which is estimated to be 25% on dry weight basis. Thus instead of storing fruit, the storage of mucilage in the form of dry powder is being recommended. Because, in the form of dry powder this alkaloid bearing part could be stored for longer period avoiding deterioration of glycoalkaloid that has been experienced during storage of fruit as a whole.

During investigation no trace of any crystal is observed in any sub-fractions of the fruit pulp i.e., rest of the fruit after separation of seed and mucilage. Though this part has long been considered as the site of biosynthesis and accumulation of glycoalkaloid in the plant.

From anatomical preparation of developing seed it appears that mucilage is formed due to degradation of outer cell layer of seed coat (extra testa) during maturity of fruit and that carpesterol is suggested for the first time to show importance in connection with the formation of highly thickned cell wall in the inner most layer of cell in the seed coat. This ultimately help the seed to produce the hard seed coat at the time of maturity. Thus out of different parts of fruits seed has been considered to have a great role on the biosynthesis and accumulation of glycoalkaloid and this may also be considered as the first time to report in this field of work.

The study on seed germination behaviour of such an important plant like Solanum viarum Dunal is very essential specially when raising of large number of seedlings is necessary for its commercial utilisation. While working on this aspect of biological phenomenon a sharp decline in the percentage of seed germination within a year after hervest has been observed. It is mainly observed during storage of seed in laboratory condition. It reaches non viability after five years of its storage. Seed deterioration is a natural catabolic process which terminates the life span of seeds, resulting in loss of viability. No work has so far been made on the changes in chemical constituents in non-viable seed due to deteriorative process involved in the seed of this plant. The petroleum ether fraction of non-viable seeds (3-years-old) shows the presence of β-sitosterol and lanosterol which are common in viable seeds. Though carpesterol like compound has been isolated from the deteriorated seed. But from mass fragmentation pattern and melting point 237-240°C it is supposed have undergone some change in molecular configuration during degradation of the seed and further study is necessary for its identification. Moreover two phenolic acid i.e., caffeic acid and Benzoic acid which are not detected in the viable seed are present in non-viable seeds. The occurrence of such phenolic acids in non-viable seeds is suggested to be due to deterioration of structural integrity within the seed during storage conditions.

The inevitable deterioration of seed is a matter of serious concern to the seed physiologists and specially in the region of North Bengal, where temperature and high relative humidity greatly accelerate seed ageing to cause a loss of huge amount of seeds every year. An attempt has been made to study the effect of various chemicals on the storage seed of *S. viarum* Dunal with a view to understanding their role on bringing about qualitative improvement in seed performance. Two types of seeds have been utilised during invigouration one is stored for 60 days and the other one for 270 days.

Out of all the chemicals taken into consideration vitamins have been observed to show maximum invigouration activity in all the two types of stored seeds. Out of which thiamine at the concentration of 10^{-5}M shows maximum of 85% of germination of seed stored for short period against control which shows only 59.7%. Similarly nicotinic acid shows maximum of 90% germination for the seed stored for longer period as compared to control showing only 10.7%. Though pyridoxine hydrochloride, pantothanic acid and thiamine have stimulated germination of seed stored for longer period but their activity of inviguration is much more when they are used in combination with IAA. Similarly the application of several amino acids like glycine, glutamic acid, aspertic acid, arginine and cystine also shows stimulation of seed germination. Out of all glutamic acid may be considered as the best as it shows maximum of 87% (10^{-3}M) and 70% (10^{-2}M) of germination of seed stored for short and long period respectively. Thus application of vitamins and amino acid may be recomended for invigouration of seed for raising of seedlings from stored seeds. Out of different flavonoids Morin and Rutin has been observed to show maximum stimulation of germination for the seed stored for short duration. But morine is much effective in connection with seed stored for longer duration. Quercetin which is the aglycone part of rutin does not show much stimulation of seed germination as compared to others.

GA has been observed to show much stimulation of germination of both the types of stored seeds. Phenolic compounds like resorcinol, salicylic acid and Tannic acid show significant stimulation in germination of seed stored for short duration, but they are not much effective for the seed stored for longer period. All these phenolic compounds show stimulation of germination for both the types of seeds when they are used in combination with GA.

Effect of growth retardants like Maleic hydrazide and Alar-b9 and metabolic inhibitors, on stimulation of seed germination has been observed. Much stimulation of seed germination has been caused by 8-azaguanine specially in connection with the seed stored of short duration. On the other hand maleic hydrazide is much effective for the seed stored for longer period. So far as the stimulation of deteriorated seed germination is concerned.

Out of several phenoxyacetic acids such as p-chlorophenoxyacetic acid, 2,4-D, 2,4,5-T; PAA and p-chlorophenoxyacetic acids have observed to show stimulation of germination of seed stored for short duration but PAA and 2,4-D show much stimulation in connection with long storage seeds. The stimulatory action is effective at the lower concentration other wise higher concentrations are inhibitory infunction.

Various sugars such as glucose, fructose, sucrose, arabinose, mannose and xylose, have shown over all stimulation of germination of seed stored either for short or long period.

Effect of different micronutrients such as manganese, copper, cobalt, nickel and boron on germination of deteriorated seeds has been observed. Manganese has been observed to be the best so far as the stimulation of germination for stored seeds. But copper and nickel have much invigouration acitivity in seeds stored for longer duration. Besides these micronutrients cadmium, lead and mercury have also been observed to stimulate germination of seed stored for short duration. But they are not so effective for long storage seeds excepting cadmium which shows significant stimulation specially at lower concentration.

Thus a number of chemicals have been screened out to show invigoration activity in deteriorated seeds of _Solanum viarum_ Dunal and which may be utilised to prevent wastage of seed being deteriorated due to ageing process.

It appears from the earlier report that special stage of development of the fruit of _S. viarum_ Dunal is necessary to show maximum yield of steroidal glycoalkaloid. In order to have better understanding of metabolism of steroidal alkaloid, investigation has been carried out to study fruit physiology with special emphasis on quantitative estimation of various biochemical parameters, in developing berries.

The rapid increase in content of soluble and insoluble carbohydrate at the initial phase of development of fruit is characteristic for S. viarum Dunal. Both the contents decline at the initial phase of development but gradually increase upto 60 days of development of fruit. In fact the "turning yellow" fruit (60 days old) has been noted to contain maximum amount of glycoalkaloid. On the other hand, total nitrogen has been noted to increase accumulation upto 30 days of development of fruit after that it gradually decline till senescent stage of the fruit. Free amino acid has also been noted to decrease gradually as the fruit attains maturity. Both the protein and phenol content gradually increase upto 60 days of development of fruit after fertilization. After that all the contents decline up to senescence of the fruit. The similar trend in rise and fall of carbohydrate as well as total glycoalkaloid content sequencing with the maturity of fruits, very much supports the idea that solasodine is a product of "high carbohydrate" rather than "high nitrogen" condition. The location of the glycoalkaloid, being the extracellular, mucilage, is considered to have a role on the protection against the loss of solasodine that commonly occur in other species where the alkaloid is intracellular in nature. The decrease in free amino acid content is associated with increase of protein and RNA upto the stage of turning yellow of fruit. This is justified for high requirement of enzyme protein during biosynthesis of high amount of glycoalkaloid. The gradual rise of phenol content upto the stage of yellowing is very much related to the biosynthesis of considerable amount of carpesterol containing benzoic acid.

The enzymatic activity in developing fruit also reflects the normal behaviour to attain senscence of fruit.

Peroxidase activity has been observed to be maximum at the initial stage of the development of fruit upto 40 days, then it gradually increase upto 50 days. After that it has been observed to decline. At the initial stage of development of fruit polyphenol oxidase activity has been noted to decline. The increase in activity has been observed upto 50 days then it decline upto senescence of the fruit. The catalase activity has been observed to be maximum at the initial stage of development fruit then it gradually declines upto 80 days.

While working on the effect of various chemicals on the growth performances of the plant, various growth parameters have been estimated at pre reproductive, reproductive and post reproductive stage of the plant. Out of all the chemicals taken into consideration, vitamins no doubt show the beneficial effect on growth and development of plant.

They are very much effective to increase the height of the plant. Maximum number of branches per plant has been produced due to treatment of p-chlorophenoxy acetic acid and -K culture. Maximum of spreading has been recorded due to treatment of nicotinic acid though beneficial effect of pyridoxine hydrochloride, $ZnCl_2$, -P and -K culture have been recorded. Production of maximum number of leaves per plant has been observed due to thiamine hydrochloride treatment though nicotinic acid and maleic hydrazide show the promising result. Significant increase of leaf area per plant has been observed due to treatment of maleic hydrazide and -K culture. So far as the dry matter accumulation in leaf is concerned -P and -K culture show the highest value.

All the three chlorophenoxyacetic acids showed morphological abnormalities in leaf specially at higher concentrations. The leaf lamina has been shown to be modified into different shapes such as spathulate, reniform etc. Veinlets have been noted to be aggregated to midvein and spines are also aggregated to the veins. Spines have been noted to occur in groups. Though reduction in leaf has occured due to treatment of all chlorophenoxy acetic acid. The increase in leaf area has been observed to be due to suppression of the main axis followed by subsequent increase of branches and sub-branches with the production of normal broad leaves giving the plant a roselte appearance.

Nicotinic acid shows the highest value in connection with the effect on dry matter production of root against complete culture. p-chlorophenoxyacetic acid has stimulated the maximum production of dry matter of stem though -K culture shows the promising result. Significant increase of total biomass production per plant has been observed in -P, -K culture and pyridoxine hydrochloride as against control. P-chlorophenoxy acetic acid has been observed to show maximum production in number of fruit though diameter of fruit and total dry weight of fruit/plant has been increased due to treatment of pyridoxine hydrochloride and $MnCl_2$ respectively. Pyridoxine hydrochloride also caused maximum production in number of seed/fruit accompained by maximum percentage of alkaloid in the fruit. The average weight of seed has occurred due to treatment of DK.

Different biochemical parameters such as chlorophyll protein and carbohydrate soluble and insoluble has been estimated from the 3rd leaf of the plant. P-chlorophenoxyacetic acid and 2-4,D has shown maximum stimulation of chlorophyll synthesis at the pre-reproductive stage, DK and MH at reproductive and DK, complete solution, MH and NA at the post reproductive stage. The chlorophyll content has been observed to show decrease in content immediately after their treatment though significant increase in chlorophyll content has been observed, at the subsequent stages of development.

Pyridoxine hydrochloride, NA, thiamine, complete solution and -P have been observed to stimulate protein synthesis of reproductive stage. Increased amount of protein has occurred at reproductive stage due to treatment of pyridoxine hydrochloride thiamine and DK. But thiamine and complete solution stimulated the same at the post reproductive stage.

Vitamins have been observed to show significant increase of carbohydrate accumulation in leaf of S. viarum at all three stages of its development.