

CHAPTER - III

EFFECT OF CHEMICALS ON SEED GERMINATION BEHAVIOUR
IN SOLANUM VIA DUNAL WITH SPECIAL EMPHASIS ON
ITS INVIGOURATION.

I N T R O D U C T I O N

The studies carried out on the germination of seed in Solanum viarum Dunal are very limited. Patil (1967) reported germination behaviour after irradiation of seeds. Vicent (1973) observed stimulation of seed germination in presence of KNO_3 solution following various storage and temperature treatment. Chauhan (1978) studied extensively the effect of ethral on germination of seed and suggested that lower concentration of ethral was stimulatory as compared to higher concentration which was inhibitory in function. Stimulation of seed germination in presence of GA was observed by Pingle and Dayan sagar (1979). Sharma et al., (1979) pointed out that low germinability was an acute problem and most of the worker had to face much difficulty due to its irratic germination behaviour as pointed out by Chatterjee (1976). The irratic seed germination behaviour was probably due to various kinds of dormancy that were overlooked by the previous authers. Later in 1980, Laha and Basu pointed out seed coat dormancy along with photodormancy operating during seed germination in the plant. They also showed that out of several chemicals GA was observed to be a potent reliever of photo_dormancy. Though some aspects of seed germination especially dormancy, stimulation and inhibition of such phenomenon have been worked out in details, no attempt has so far been

made on the study of deteriorated seeds.

Rapid deterioration leading to non-viability of seeds is a serious problem in tropical and subtropical countries like India and specially in North Bengal where high temperature and high relative humidity greatly accelerate seed ageing phenomenon resulting in their deterioration. Seed of S. viarum Dunal in particular are very sensitive to these adverse climatic conditions and often becomes subjected to rapid deterioration under ambient storage environment creating serious problems for plant growers of the region. Huge amount of seeds are being lost each year.

Here an attempt has been made to study the effect of various chemicals on the harvested and deteriorated seeds of S. viarum Dunal with a view to understanding their role on bringing about qualitative improvement in seed performance. The study will be helpful to the farmers for utilisation of improved seeds in connection with cultivation of the plant for commercial purpose.

MATERIALS AND METHODS:

MATERIALS :

Solanum viarum Dunal ($2n = 24$) was cultivated in the experimental plot of the Centre for Life Sciences, North Bengal University and seeds were collected from the mature

fruit at the brown stage and utilised during the investigation on seed germination.

After sun drying, seeds were kept in the glass bottle fully closed with the bakelite cap. They were stored in the laboratory keeping at the room temperature. Two types of stored seeds were used.

i) 60 days stored and ii) 270 days stored seeds.

METHODS :

Scarification of seed :

Chemical scarification of seed was performed with 80% H_2SO_4 for 5 minutes. Excess acid was decanted off and a large volume of water was poured. The scarified seeds were then washed thoroughly in running tap water and finally with distd. water and placed on moist cotton pad and set in different petridishes.

Germination of seeds :

During germination study, scarified seeds were washed thoroughly. They were pre-soaked in respective chemicals for eighteen hours and were placed on moist cotton wool in petridishes. Five sets of petridishes, each containing 100 seeds were prepared and replicated. The cotton wool was soaked with respective chemical solution. Petridishes containing seeds were placed under flourescent light (200 lux) at $26^{\circ}C \pm 1^{\circ}C$. Distilled water was added every alternate day to maintain the

constant moisture level in each set. Seeds were considered to have been germinated when emerging radical was easily observed and the percentage of germination was recorded every day. Final observation was recorded on thirteenth day after initiation of the experiment.

Preparation of chemical solution :

Stock solution (1M) were prepared for each of the following chemicals used to observe their effect on seed germination. From the stock solution 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} and 10^{-7} M were produced and utilised during seed germination study.

IAA of 10^{-5} M was used in combination with vitamins.

GA of 10^{-1} M was used in combination with phenols.

Unless other wise stated scarified seeds in distilled water was served as control. Following chemicals were used during seed germination study.

Vitamins :

Pyridoxine hydrochloride, Nicotinic acid, Pantothenic acid, Thiamine hydrochloride, Riboflavin and Biotin.

Phenoxy acetic acid :

Phenoxy acetic acid, p-chlorophenoxy acetic acid, 2,4 - Dichlorophenoxy acetic acid, 3,4-dichlorophenoxy acetic

acid, 2,3,5-trichlorophenoxy acetic acid, and 2,4,5-trichlorophenoxy acetic acid.

Micronutrients :

H_3Bo_3 , $MnCl_2$, $CuCl_2$, $ZnCl_2$, $CoCl_2$ and $NiCl_2$.

Amino acids :

Glutamine , Glycine, Aspartic acid, Arginine and Cystine.

Phenols :

Resorcinol, Salicylic acid and Tannic acid.

Growth regulators :

Rutin, Morin, Quercetin, Alar -b₉ ; 8-Azaguanine, Maleic hydrazide and Dikegulac - sodium.

Growth hormone :

Indole acetic acid and Gibberelic acid.

Sugars :

Glucose, Fructose, Sucrose, Arabinose, Mannose and Xylulose.

Heavy metals :

Mercuric chloride, Lead chloride and Cadmium chloride.

TTC test :

To perform TTC (2,3,5-triphenyl tetrazolium chloride) seeds were soaked for 8 hours in deionised distilled water. Seed coat was then removed and immersed in 1% TTC solution. The rose red formazon was extracted following the method of Powell and Mathews (1977) after taking to embryonal axes with three replicates in 10 ml. of 95% ethanol (v/v) at 80°C for five minutes. After cooling the absorbance of the extract was measured in a spectrophotometer (C.Z. Instruments) at 520 nm. Rating of seeds on the basis of variation of TTC staining was done visually and catagorised as follows :

- I. Whole seeds (cotyledons) deeply coloured.
- II. Embryo deeply coloured and deep patches on cotyledons.
- III. Embryo and cotyledons lightly coloured.
- IV. No colouration.

Statistical analysis :

Statistical analysis on the data has been worked out in collaboration with the Department of Computer Centre, North Bengal University, following the Method utilised by Goon Gupta and Das Gupta (1978).

Tou (γ) value has been calculated according to the formula :

$$\gamma = \frac{(\bar{x} - \mu_0) \sqrt{n}}{6}$$

Where \bar{x} = Sample mean, μ_0 = mean of control, σ = standard deviation of control. n = number of tests have n = 5.

RESULT AND DISCUSSION

Seed deterioration is a natural catabolic process which terminates the life span of seed, resulting in loss of viability. The process may be accelerated externally by some pathogenic attack or by adverse environmental condition otherwise the deteriorative events may follow their normal course culminating in the production of non-viable seeds. This inevitable deterioration of seed is a matter of serious concern to the seed physiologists and various strategies are being developed to prolong the storage potentiality of seeds.

Reduced seed germinability as well as slower rate of germination are considered to be important visible criteria for the evaluation of poor seed vigour (Anderson, 1970 ; Abdul-Baki and Anderson, 1972). But with the help of seed germinability it is very difficult to distinguish between non-viability and dormancy of seed. According to Mayer and Polijakoff-Mayber (1978) all histochemical methods devised to test viability are based on the activity of certain oxidising enzyme and the best correlation has been found to be the activity of enzyme reacting with redox dyes such as tetrazolium.

The seed of S. viarum Dunal stored for a longer period attains the non-viability and which has been experimentally demonstrated with tetrazolium test. Table-16 shows that the optical density of formazon colour complex in the cotyledons and embryo gradually decreased along with decreased percentage of seed germination due to prolonged storage condition. Seeds have been noted to attain non-viability after one year. This is very much contradictory to the observation of earlier worker who claimed that the seed of S. viarum Dunal became non-viable during 2 to 3 months after harvest and that the seed possessed a dormancy for one month immediately after harvest. But our experiment suggest that seeds of S. viarum Dunal obtained from freshly harvested yellow fruit as well as dried brown berries have the ability to germinate if proper environmental conditions are available and the seed coat dormancy is to be considered as primary factor to interfere with the germination. While working on the effect of certain chemicals on storage seed to stimulate germination it has been noted that at least a number of amino acids such as glycine, glutamic acid, aspartic acid show stimulation of germination showing maximum of 75.5%, 87.2% and 72.4% respectively for shortly stored seeds (Table 17) and they have been observed significant statistically at 1 percent level. All these chemicals have also been shown to stimulate germination over control. Specially with the seed stored for longer duration (table-17). The result has also been to be significant at 1 percent

Table - 16

Percentage of seed germination during storage and TTC test on the viability of seeds in S. viarum Dunal.

Month of seed germination	% of seed germination	Age of the harvested seeds (in month)	Rating category	OD. values of formazon colour complex
January(1989)	81 ± 2	1	I	0.19
February	80 ± 3	2	I	0.18
March	82 ± 2	3	I	0.15
April	79 ± 3	4	I	0.14
May	62 ± 1	5	I	0.12
June	50 ± 2	6	II	0.09
July	41 ± 2	7	II	0.06
August	25 ± 3	8	III	0.05
September	19 ± 1	9	III	0.03
October	15 ± 2	10	III	0.02
November	11 ± 1	11	III	0.02
December	2 ± 1	12	III	0.01
January(1990)	-	13	IV	-

level.

Though information is available in connection with the role of amino acid in relation to metabolism of germinating seed but their involvement in connection with the mechanism of stimulation of deteriorated seed from biochemical point of view is not known, though Zolotov (1965) observed increase of growth vigour and germination of seed of sugar beet due to the treatment of 5 to 10% of glutamic acid.

The loss of vital cellular components during seed aging is well documented (Abdul-Baki and Anderson, 1972). Increase in soluble substances such as sugars and amino acids during the process of seed deterioration has also been shown (Ching and Schoolcraft, 1968 ; Pearl et al., 1978). In their experiment they reported that increase of protease activity occurs under accelerated aging with concomitant reduction in the activities of other enzyme. Though in S. viarum Dunal seed attains non-viability but the reason for stimulation of seed germination due to additional supply of certain amino acid inspite of the fact that the storage seed contain considerable amount of free amino acid.

Anderson (1977) assayed RNA and protein synthesis in axes excised from dry soybean (Glycine max L.) at different levels of deterioration and observed that lower rates of RNA and protein synthesis in deteriorated seed were associated

with reduced ATP content of the tissue. He also noted that ribosomes and water soluble components of partially deteriorated soyabean seeds appeared to be as effective as those of sound seeds in supporting protein synthesis. That ATP is synthesised from C^{14} glycine in isolated soyabean embryonic axes, following the metabolic pathway of purine nucleotide synthesis was confirmed by Anderson (1979).

Recently polyamino content and the activity of certain enzymes relating to polyamino metabolism has been noted to change significantly in germinating seed (Altman et al., 1983). Galston (1983) showed that in many cases growth inhibitions could be reversed by polyamino content and that it increases cell division. Smith (1982) critically discussed the function and metabolism of polyamines in higher plants and according to Galston (1983) three amino acids, the poly-basic amino acids, arginine and lysine furnish the main part of the carbon skeleton of polyamines while methionine contribute polyamino groups to the simple diamine to form polyamines. There is now evidence that natural polyamine stabilises cell membrane and retards senescence (Slocum et al., 1984) and influence natural and stress induced senescence (Altman, 1977, 1982).

Thus there is a possibility of exogenously applied amino acid to deteriorated seeds of S. viarum Dunal by the change of polyamine content and which is yet to be worked out. Recently Basu and Gupta (1988) observed that Tephrosia candida,

a nitrogen fixing leguminous plant and a native plant of North Bengal played an important role on the increase of amino acid content in the soil of North Bengal. Moreover during field trial Solanum viarum Dunal has been observed to show the best result in growth performance while it is grown in association with F. candida. The allelopathic effect may be due to liberated amino acid by leguminous plant in the soil.

In connection with the treatment of different vitamins on seed germination behaviour in S. viarum Dunal (table 18) it has been noted that pyridoxine hydrochloride showed stimulation of seed germination at all the concentrations (10^{-2} to 10^{-7} M) out of which 10^{-4} M solution showed 73.5% of seed germination as compared to control (59.7%). Similar observation was also noted in connection with nicotinic acid which showed maximum of (72.4%) seed germination at 10^{-5} M. (92.6%) of seed germination was observed at 10^{-4} M concentration of thiamine hydrochloride. Nicotinic acid and pantothenic acid stimulated germination at the concentration of 10^{-5} M, showing maximum of (72.5%) and (66.5%) respectively with the lower rate of germination at the higher and lower concentration of the solution in both the cases. Ascorbic acid and Biotin showed stimulation of it at the lower concentration of 10^{-7} M only showing 62.2% and 66.5% as compared to control (59.7%). All these values have been observed to be significant statistically at 1 percent level (table-18).

In connection with the much deteriorated seeds all vitamins showed stimulation of seed germinations. Nicotinic acid is remarkable in the sense that it triggered upto 90% of seed germination at 10^{-2} M as compared to control which showed only 10.7%. This vitamin has been observed to show stimulation at comparatively higher concentration as compared to biotin which was shown to be effective at the lower concentration showing maximum of 53.4% at 10^{-7} M soln. Ascorbic acid showed maximum of 24.2% of seed germination at the concentration of 10^{-7} M (table-17). Stimulation of nicotinic acid in the seed of wheat, barley, oat and corn during germination has been shown by several workers (Burkholder and Mcveish, 1942 ; Burkholder, 1943 ; David et al., 1943 ; Alvin, 1950). The results of Ghosh et al., (1963 a,b) demonstrated that nicotinic acid is stored in the cereals as niacinogen from which it is released by a hydrolysing enzyme during germination and is subsequently utilised for the synthesis of NAD. Yemm and Willis (1956) suggest that NADH made available from the reduction of NAD during glycolytic breakdown of sugar is utilised either in the synthesis of amino acids by reductive amination of keto acids or in other amino acid inter conversions by transaminases. Also the some of NADP and NADPH is greater in the embryo which have a high synthetic activity than in storage tissue which have a relatively low synthetic activity (Klingenberg and Bucher, 1960 ; Lowenstein, 1960).

Seeds of different plant contain different quantities of vitamins. The quantity of these vitamins in seeds of plants from one and the same variety grown under different conditions also differ (Ovcharov, 1977). Therefore, it is understandable that under certain conditions seeds accumulate the quantity of vitamins necessary for their germination while under other conditions the vitamin content is insufficient.

Medir (1964) first pointed out that in non germination seeds of various plants significantly low quantities of vitamin and amide of nicotinic acid occurred, and according to Ovcharov (1977) vitamin content of seeds drops due to prolonged storage. Sulakadze et al., (1955) observed significantly increased percentage of germination by the treatment of seed with nicotinic acid and thiamine. Similar observation was recorded by Zakhar'yants et al., (1950). It has been demonstrated by Ovcharov et al., (1966) that seeds of maize at different physiological stages of ripeness responded differently to an additional supply of biotin. Wheat seeds showed different germination capacities in presence of 0.01% solution of ascorbic acid and a 0.001% solution of glutamic acid led to intensive synthesis of auxins (Skrabka, 1965). The germination of wheat seeds stored for 9 years was increased after soaking them in copper sulphate in a solution of glutamic acid or in a mixture of glutamic acid, ascorbic acid and copper sulphate for 14 hrs.

Billi, (1954) suggests that pyridoxine participates in the biosynthesis of the γ -amino butyric acid that forms at the time of seed germination. With a deficiency in this vitamin many aspects of amino acid synthesis are disturbed. With the enrichment of seeds and seedlings with this vitamin the metabolic process is normalized.

Thus it is possible to conclude that seed of S. viarum Dunal during storage suffers from a deficiency in some vitamins and the timely supply of the requisite vitamins to such seeds intensifies metabolism and improves their germination.

IAA has been found to have both stimulatory as well as inhibitory activity in connection with seed germination. The lower concentration (10^{-5} M to 10^{-7} M) increased percentage of germination (75% at 10^{-6} M) as compared to control (59.7%) in connection with shortly stored seeds while the range of 10^{-3} M to 10^{-4} M showed stimulation of germination in much deteriorated seeds, (32% to 55.6%) as compared to control (10.7%). Higher concentration (10^{-2} M) and the lower concentration (10^{-6} M to 10^{-7} M) showed inhibitory effect (table-18).

The effect of IAA on germination has long been a matter of dispute (Mayer-Poljakoff-Mayber, 1978). Various workers investigated the effect of IAA on germination of a variety of seeds and obtained conflicting result, stimulation

and inhibition being obtained depending on the concentration of IAA and the type of seed.

Experiments have been conducted to show the effect of IAA in combination with different types of vitamins. Table-19 shows that there was marked synergistic effect of vitamins along with IAA in case of deteriorated seeds as compared to the data (table-18) obtained when only vitamins were used not in combination with IAA. As for example pyridoxine hydrochloride when used singly showed only 54.2% at 10^{-3} M. But 71.6% of seed germination was observed when the same concentration of vitamin was used along with IAA (10^{-5} M) specially in case much deteriorated seeds. Similarly maximum of 38% was observed when pantothenic acid (10^{-2} M) was used as compared to 78.3% of seed germination when it was used in combination with IAA.

It is well known that gibberellins are involved in seed germination. Exogenous gibberellins enhanced the germination of both negatively (Chen and Thiman, 1966) and positively (Karrsen, 1976) photoblastic seeds. It is suggested that the annual seeds accumulate "stored" GA which become active during imbibition and trigger germination (Pressman et al., 1988 ; Pressman and Shaked, 1988). The reversal by exogenous GA of almost complete inhibition of seed germination due to presence of tetracyclisis and triazoles, confirms the validity of concept (Kepezynski, 1986 ; Hallaham et al., 1988).

Table - 19

Synergistic effect of IAA and Vitamins (Pyridoxine hydrochloride, Pantothenic acid and Thiamine HCl.) on seed germination of S. viarum Dunal.

Treatments	Conc. (M)	% of germination	
		(60 days)	(270 days)
I A A	10 ⁻²	50.5(-3.671) <	4.0(-7.136) <
	10 ⁻³	55.0(-1.875) <<	55.6(47.819) *
	10 ⁻⁴	58.8(-0.559))	32.0(22.684) *
	10 ⁻⁵	63.5(11.516))	10.5(-0.213))
	10 ⁻⁶	65.6(2.354) *	14.2(-6.923) *
	10 ⁻⁷	75.0(6.105) *	16.4(6.070) *
Pyridoxine hydrochloride + IAA	10 ⁻²	55.4(-1.716) <<	31.6(22.259) *
	10 ⁻³	68.8(3.631))	71.6(64.859) *
	10 ⁻⁴	86.0(10.494) *	56.0(48.244) *
	10 ⁻⁵	82.6(9.137) *	32.6(23.323) *
	10 ⁻⁶	79.4(6.678) *	30.0(20.554) *
	10 ⁻⁷	64.4(1.593))	26.0(16.294) *
Pantothenic acid	10 ⁻²	50.6(-3.631) <	78.3(71.994) *
	10 ⁻³	60.4(0.279))	68.3(61.344) *
	10 ⁻⁴	86.0(10.494) *	50.0(41.864) *
	10 ⁻⁵	78.0(7.302) *	35.0(25.880) *
	10 ⁻⁶	85.0(10.095) *	27.0(17.360) *
	10 ⁻⁷	63.4(1.476))	20.0(9.904) *
Thiamine + IAA	10 ⁻²	45.4(-5.706) <	51.0(42.920) *
	10 ⁻³	65.0(2.115) **	62.6(55.273) *
	10 ⁻⁴	85.8(10.414) *	45.0(36.530) *
	10 ⁻⁵	79.2(7.780) *	41.6(32.909) *
	10 ⁻⁶	75.0(6.105) *	36.0(26.944) *
	10 ⁻⁷	62.0(0.918))	22.0(12.034) *
Control	0+0	59.7 ± 5.6	10.7 ± 2.10

* Significantly greater than control at 1% level of significance.
 ** " " " " 5% " "
 Significantly lesser than control at 1% level of significance.
 " " " " 5% " "

Table-20 shows that out of three flavonoids morin and rutin show maximum stimulation of seed germination (95.2% at 10^{-3} M) and (95.5% at 10^{-4} M) respectively. Quercetin which is the aglycone part of rutin does not show much stimulation of seed germination as compared to others. It is effective only at the lower concentration showing maximum of 62.2% of seed germination at 10^{-7} M solution as compared to control (59.7%), the result of which has been observed to be not significant statistically as compared to other chemicals (table-20).

Furuya et al., (1962) identified kaempherol, quercetin, and their glycoside to be responsible for regulation of growth in plant significantly. IAA is supposed to be a controlling factor during seed germination and seedling growth (Mayer and Poljakoff - Mayber, 1978 ; Nitsch and Nitsch, 1962). Furuya et al., (1962) suggested that monohydroxy phenol such as kaempherol is cofactor for stimulation of IAA oxidase activity while dihydroxy phenol such as quercetin is inhibitory in nature and levels of these flavonoids are under phytochrome control (Galstone, 1962).

According to Stenlid and Saddik (1963), a free hydroxyl group at 7 - position impaired the cofactor nature of the flavonoid and that is why quercetin and rutin (rutinoside of quercetin) all having hydroxyl group at the 7-position shows stimulation of germination. It is expected that flavonoid

Table - 20

Effect of Morin, Rutin and Quercetin on seed germination of S. viarum Dunal.

Treatments	Conc. (M)	% of germination	
		(60 days)	(270 days)
Morin	10 ⁻²	45.2(-6.612) <	4.0(-13.614) <
	10 ⁻³	95.2(16.188) *	6.0(-9.550) <
	10 ⁻⁴	85.0(11.537) *	7.2(-7.112) <
	10 ⁻⁵	77.0(8.117) *	8.5(-4.470) <
	10 ⁻⁶	73.2(6.156) *	9.6(-2.235) <<
	10 ⁻⁷	72.0(5.609) *	10.8(0.203))
Rutin	10 ⁻²	40.8(-8.618) <	6.0(-9.550) <
	10 ⁻³	75.2(7.068) *	8.2(-5.080) <
	10 ⁻⁴	95.5(16.324) *	7.8(-5.893) <
	10 ⁻⁵	80.6(9.530) *	9.8(-1.829) <<
	10 ⁻⁶	76.4(7.615) #	10.0(-1.422)
	10 ⁻⁷	75.0(6.977) *	11.0(0.610))
Quercetin	10 ⁻²	40.2(-8.892) <	5.0(-11.582) <
	10 ⁻³	50.2(-4.332) <	8.2(-5.080) <
	10 ⁻⁴	51.5(-3.739) <	8.5(-4.470) <
	10 ⁻⁵	55.6(-1.870) <<	10.0(-1.422)
	10 ⁻⁶	60.4(0.319))	10.2(-1.016)
	10 ⁻⁷	62.2(1.14))	11.5(1.626))
Control	0±0	59.7 ± 4.9	10.7 ± 1.1

* Significantly greater than control at 1% level of significance
 ** " " " " 5% " "
 Significantly lesser than control at 1% level of significance
 " " " " 5% " "

may have certain role on seed germination and growth physiology of Solanum viarum Dunal with the content of IAA regulated by IAA oxidase activity.

GA was observed to show much stimulation of germination of both types of stored seeds (table-22). Phenolic compounds like resorcinol, salicylic acid and tannic acid showed significant stimulation in seed germination specially after shortly storage condition of the seed but they were not much effective in connection with seeds stored for longer

period. All these phenolic compound showed stimulation of germination for both the types of seed when they were used in combination with GA (table-21,22). The regulation of plant growth is determined by the balance between phytohormone and phenolic inhibitors produced in time and space. Polyphenols influence growth and development of higher plants by interacting with auxin and gibberellic acid (Green and Corcoram, 1975; Jacobson and Corcoram, 1977) and with cyclic Amp (Kwon et al., 1978 a,b). Information (Bhalla and Sabharwal, 1975 ; Khurana and Maheshwari, 1978) is available in connection with the participation of phenols in plant metabolism as chelating agents.

Laloraya (1980) has shown that polyphenols could antagonize the gibberellin induced growth in pea and lettuce. Besides gibberellins, phenols antagonized the strong inhibitory effects of abscisic acid on seedling growth and some other

Table - 21

Effect of Monophenol, Diphenol and Polyphenol on seed germination of S. viarum Dunal.

Treatments	Conc. (M)	% of germination	
		(60 days)	(270 days)
Resorcinol	10 ⁻²	45.2(-5.887) <	5.0(-6.709) <
	10 ⁻³	55.0(-1.908) <<	9.8(-1.059)
	10 ⁻⁴	63.0(1.334)	9.5(-1.412)
	10 ⁻⁵	70.2(4.263)*	10.0(-0.824)
	10 ⁻⁶	75.4(6.374)*	10.8(0.118)
	10 ⁻⁷	73.0(5.400)*	13.0(2.707)*
	Salicylic acid	10 ⁻²	18.2(-16.849) <
10 ⁻³		20.1(-16.078) <	4.0(-7.886) <
10 ⁻⁴		50.5(-3.735) <	10.5(-0.235)
10 ⁻⁵		56.2(-1.421)	12.4(2.001)**
10 ⁻⁶		58.4(-0.528)	13.0(2.707)*
10 ⁻⁷		63.0(1.340)	15.0(5.061)*
Tannic acid		10 ⁻²	60.2(0.203)
	10 ⁻³	66.4(2.720)*	9.0(-2.001) <<
	10 ⁻⁴	72.5(5.197)*	10.0(-0.824)
	10 ⁻⁵	68.8(3.695) *	10.0(-0.824)
	10 ⁻⁶	70.5(4.385)*	11.0(0.353)
	10 ⁻⁷	75.6(6.455)*	12.5(2.119)**
	Control	0±0	59.7 ± 5.5

* Significantly greater than control at 1% level of significance
 ** " " " " 5% " "
 * Significantly lesser than control at 1% level of significance
 ** " " " " 5% " "

abscisic acid mediated responses (Apte and Laloraya, 1982). Maximum reversal was obtained at low concentration of phenol (10^{-5} to 10^{-7} M) which suggested that the formation of GA_3 phenol complex may explain a number of observations in literature concerning biosynthesis and metabolism of gibberellin and growth regulating properties of phenols. Kefeli and Kutacek (1977) reached a conclusion that phenol interfere not only with the action of auxin but also with other hormones.

The action of phenolic compounds on plants growth is frequently attributed to their interaction with IAA oxidase, thus regulating IAA levels in vivo (Schneider and Wishtman, 1974 ; Thiman, 1972 ; Vansumere et al, 1975 ; Wolf et al., 1976 and Letham, 1978). In general it was postulated that monophenol stimulate IAA decarboxylation and thus inhibit growth whereas dihydroxy phenolics inhibit IAA oxidation to stimulate growth.

Phenolics are also reported to regulate the uptake of K^+ , Ca^{++} and iron uptake (Muthu Kumar et al., 1985).

In connection with the effect of growth retardants like maleic hydrazide, alar-b9 and metabolic inhibitor like azaguanine on seed germination behaviour of Solanum viarum Dunal much stimulation on seed germination has been observed specially in connection with the seed stored for short period

(table-23). But in connection with the seeds stored for longer period, Maleic hydrazide has been observed to be much effective so far as the stimulation of seed germination is concerned (table-23). Growth retardants like maleic hydrazide, alar - b9 and metabolic inhibitor like azaguanine produced inhibition of germination and the extent of inhibition decreased with increase in storage period (Gupta, 1970), while GA_3 partially relieved the germination inhibition caused by maleic hydrazide and azaguanine. It failed to relieve the same when the seeds were treated with alar-b9. Inhibition of germination by alar - b9 was more alternated when it combines with gibberellic acid. Explanation for such an interaction between a germination promoter and an inhibitor resulting to a synergistic expression of inhibition might to be sought in the observation of Moore (1967) who observed that biochemical action of alar - b9 was different from other growth retardants.

In the literature dealing with seed viability, there are some reports that hydration - dehydration treatment as well as treatment of seeds with chemicals of diverse nature (Salts, phenols, organic acids etc.) can favourably influence the viability status of seeds (Basu et al., 1979 ; Savino et al., 1979 ; Pathak and Basu, 1980). Recently, prolongation of seed vigour and viability under storage condition by seed pretreatment with dikegulac-sodium has been shown (Bhattacharjee,

1984 ; Bhattacharjee and Gupta, 1985 ; Bhattacharjee et al., 1986 ; Bhattacharjee and Choudhuri, 1986 ; Bhattacharjee and Bhattacharjee, 1986). According to them whatever might be the exact mechanism behind it there is a least doubt to infer that dikegulac can enhance the storage potential of seed, there by retaining seed vigour and viability for longer duration. Table-23, shows that dikegulac-sodium stimulated germination of seed stored for longer duration at all the concentrations as compared to other growth retardants which showed much inhibition of seed germination at higher concentration. Maximum of 78.4% of seed germination was observed due to treatment of dikegulac at 10^{-3} M as compared to 95.6% observed due to effect of maleic hydrazide (10^{-4} M) and azaguanine (10^{-3} M) when control value was only 59.7% for the seed stored for short duration.

In connection with the effect of different types of phenoxy acetic acid the table-24 shows that they are inhibitory in action excepting PAA and p-chloro phenoxy acetic acid which showed maximum stimulation of seed germination of 65% (10^{-7} M) and 70% (10^{-4} M) respectively as compared to control (59.7%). So far as the deteriorated seeds for longer storage is concerned PAA and 2,4-D showed certain amount of stimulation showing maximum value of 12.4% and 15.4% for PAA (10^{-7} M) and 2,4-D (10^{-7} M) respectively as compared to control (10.7%). The stimulatory action was effective at the lower concentration otherwise higher concentrations were inhibitory in function.

The effect of different types of phenoxy acetic acid on metabolism in general has not been studied very extensively except for a few cases. The compound most widely investigated has been 2,4-D. According to Mayer and Polijakoff-Mayber (1978) 2,4-D induces profound changes in the metabolism of treated plants. These occur in the metabolism of nitrogenous compounds, carbohydrates and to some extent respiration. Many enzymes have been shown to be affected to a greater or lesser extent by 2,4-D. In germinating lettuce and wheat, changes in carbohydrates metabolism occurs after 24 hours of treatment. Respiration of wheat and lettuce increased somewhat by 2,4-D and this increase was accompanied by changes in respiratory quotient. 2,4-D treatment actually increases the amount of enzymes which participate in the pentose cycle (Black and Humphreys, 1960).

Sugars are one of the metabolic components which are used by the young embryo at the time of its resumption of growth at early period of germination as in the case of all growing tissues and cells. In connection with the effect of various sugars on seed germination behaviour, majority of the sugars were noted to have stimulatory in function. Arabinose showed maximum of stimulation (95.3%) at 10^{-4} M solution as compared to control (59.7%). Maximum of 85.2% and 85.8% stimulation were observed in connection with the effect of sucrose (10^{-4} M) and fructose (10^{-4} M) respectively though mannose showed

90.5% of seed germination at the concentration of 10^{-5} M. Sugars have also been noted to stimulate germination of deteriorated seeds stored for longer period at different concentrations of solutions. Maximum of 22.2% of seed germination was observed by the treatment of mannose and arabinose at the concentration of 10^{-5} M and 10^{-3} M respectively as compared to control which showed only 10.7% of germination. Glucose stimulated upto 20% of germination when maximum of 18.2% and 17.2% caused by fructose (10^{-3} M) and sucrose (10^{-4} M) respectively were observed (table-25).

Similar experiment was also conducted by Monhot (1989) who showed stimulation of seed germination of Acacia senegal in presence of sugar such as sucrose, glucose, fructose, maltose, and galactose at 0.1% concentration. She also observed inhibition of seed germination in Leucaena leucocephala whereas in case of Parkinsonia aculeata there was initial stimulation but after wards inhibition was noted.

Embryos that have completed organogenesis (mature embryos) but have failed to germinate in intact seeds under favourable germination conditions (Presumably due to deterioration of inherent germination ability) have been reported to germinate successfully in culture media specially in presence of sucrose. (Bhattacharjee and Sen-Mandi, 1985). Davis (1983) studied growth of excised embryonic axes on different

sugar. He observed that growth of mature, non-germinating (due to insufficient pregermination imbibition) embryos of pea when cultured aseptically in hormone free medium was reported to respond to different concentrations of sucrose in the medium. Recently Das and Sen - Mandi (1988) observed that sucrose alone was enough to bring about root development in non-germinating deteriorated (aged) wheat embryos.

Table-26 shows that out of different micronutrients such as manganese, copper, cobalt, nickel and boron, manganese stimulated significantly showing maximum of 95.2% germination of shortly stored seeds as compared to control (59.7%) though copper, nickel, boron and cobalt showed maximum of 90.2%, 80.4%, 82% and 73.2% respectively. But copper was very much effective for long storage seed. It stimulated seed germination at all concentrations and showed maximum of 21% at 10^{-3} M as compared to control showing only 10.7%. In this respect nickel is comparable to copper as it showed the same percentage of seed germination though it became toxic at 10^{-2} M. Manganese, cobalt and boron also stimulated germination of same kinds of seed at all the concentration excepting cobalt which inhibited the same at higher concentration (10^{-2} M).

Manganese is activator for the enzymes catalyzing the various stages of respiration. It is also associated with enzymes involved in nitrogen metabolism (Shankar, 1984 ;

Table - 26

Effect of micronutrients (Mn, Cu, Co, Bo and Ni) on seed germination of Solanum viarum Dunal.

Treatment	Conc.(M)	% of germination	
		(60 days)	(270 days)
Manganese chloride	10 ⁻²	91.6(11.516)*	15.0(5.061)*
	10 ⁻³	95.2(12.816)*	18.4(9.063)*
	10 ⁻⁴	93.0(12.021)*	19.6(10.475)*
	10 ⁻⁵	85.0(9.133)*	16.0(6.238)*
	10 ⁻⁶	78.2(6.679)*	15.1(5.179)*
	10 ⁻⁷	69.0(3.357)*	12.0(1.530)
Cuprus chloride	10 ⁻²	74.6(5.379)*	20.2(11.181)*
	10 ⁻³	90.2(11.010)*	21.0(12.123)*
	10 ⁻⁴	82.4(8.195)*	17.6(8.121)*
	10 ⁻⁵	76.4(6.029)*	16.0(6.238)*
	10 ⁻⁶	75.0(5.523)*	15.0(5.061)*
	10 ⁻⁷	67.2(2.708)*	15.2(5.297)*
Cobalt chloride	10 ⁻²	45.0(-5.307)<	5.0(-6.709)<
	10 ⁻³	73.2(4.874)*	12.4(2.001)**
	10 ⁻⁴	67.2(2.708)	17.2(7.651)*
	10 ⁻⁵	65.4(2.058)**	15.6(5.767)*
	10 ⁻⁶	64.0(1.552)	15.0(5.061)*
	10 ⁻⁷	61.0(0.469)	14.6(4.590)*
Boric acid	10 ⁻²	77.4(6.390)*	18.0(8.592)*
	10 ⁻³	82.0(8.050)*	15.4(5.532)*
	10 ⁻⁴	74.2(5.234)*	16.2(6.474)*
	10 ⁻⁵	68.2(3.069)*	16.0(6.238)*
	10 ⁻⁶	65.4(2.058)**	14.8(4.826)*
	10 ⁻⁷	62.4(0.975)	13.5(3.295)*
Nockel chloride	10 ⁻²	75.0(5.523)*	0.0(-12.594)<
	10 ⁻³	80.4(7.473)*	21.0(12.123)*
	10 ⁻⁴	78.4(6.751)*	18.2(8.828)*
	10 ⁻⁵	69.0(3.357)*	17.0(7.415)*
	10 ⁻⁶	65.2(1.986)**	17.0(7.415)*
	10 ⁻⁷	63.5(1.372).	15.0(5.061)*
Control	0+0	59.7 ± 6.2	10.7 ± 1.9

* Significantly greater than control at 1% level of significance
 ** " " " " " " 5% " "
 < Significantly lesser than control at 1% level of significance
 < " " " " " " 5% " "

Nason et al., 1954). The participation of manganese in the metabolism of auxins is of considerable interest (Cooil, 1952; Thiman, 1956). Sabbakh (1973) concluded that Mn^{2+} play an important role in the mechanism of action of indole acetic acid related to cell growth. Siegel and Galston (1955) have shown that manganese is essential for both the enzymatic and non-enzymatic breakdown of indole acetic acid in vitro. These ions stimulate the decarboxylation of indole acetic acid i.e., they stimulate IAA oxidase activity both in vivo and vitro (Tomaszewski and Thiman, 1966). Since manganese can stimulate growth induced by naphthylacetic acid and 2,4-D, the authors believe that the general effect of this element on growth is not connected with its direct role in auxin metabolism. According to Vlasyuk et al., (1973) manganese deficiency leads to a loss of control over the differentiation processes as a consequence of the suppression of certain regions of the DNA. Manganese ions activates the synthesis of various histone fractions (Llimovitskaya et al., 1975). Bakardjieva and Jordanov (1967) studied the effect of manganese on the oxidation of ascorbic acid. Their results also indicated that free manganese ions can fulfil the function of a peroxidase and thereby promote peroxidase dependent breakdown of indole acetic acid.

Copper also plays a role in auxin metabolism. Erkama (1950), and Ostrovakaya (1956) reported a direct

dependence of germination vigour in cereal and legume seeds on copper content. Ostrovaskaya (1956) found that copper deficiency could be alleviated by treating the seeds with indole acetic acid, which significantly enhanced germination of the copper deficient seeds. No such seed effect could be observed in seeds showing normal copper levels on treatment with copper salt solutions. A comparison of these findings with reports of the acceleration of germination in seeds having a high auxin content led Gamayunova (1965) to consider that copper is concerned in the metabolism of growth substances. Other investigator (Vanyugina, 1959) reported a positive correlation between the indole acetic acid content of plant tissues and the activity of a copper enzyme, ascorbate oxidase. Gamayunova (1965) found that the tryptophan content was greatest in copper enriched seeds during their germination. From this, she deduced that one of the functions of copper in seed germination was to play a part in process leading to an enhancement of tryptophan synthesis, tryptophan being a precursor of indole acetic acid. For the biosynthesis of ethylene from methionine ; a copper containing enzyme and O_2 are essential. Ethylene rapidly inhibits lateral auxin transport (Burg, 1973). The various physiological functions of copper in plants are intimately associated with interactions that takes place between copper and other mineral nutrients (Shokolnik and Makarova, 1950).

Cobalt ions were found to promote the incorporation of amino acids into isolated ribosomes of peas and like divalent ions of manganese, calcium, magnesium and cobalt positively influenced the interaction between aminoacyl, RNA and ribosomes (Hershko et al., 1961) one of the important physiological functions of cobalt is its participation in respiration and energy metabolism (Danilova, 1961 ; Yagodin, 1970). Evidence has been obtained on the inhibition by cobalt of ethylene synthesis at the point of conversion of methionine into ethylene (Abelles, 1973). It can activate the synthesis of fatty acids and can affect the oxidation of keto acids, unsaturated fatty acids and organic acids (Shkolnik, 1984).

Boron, which is not metal and unlike other metallic trace elements, is not an enzyme activator and plays a highly specific role in plant life processes on account of its unique significance in phenol and lignin metabolism and due to its influence on the state of membranes (Shkolnik, 1984). According to various workers (Shkolnik and Kopmane , 1970 ; Alekseyeva, 1971) boron deficiency is accompanied by a decrease in the content of various phospholipids. Pollard et al., (1977) suggests that, the specific activity of membrane components is regulated by boron, and boron plays a primary role on the control of membrane permeability. Tanada (1974) suggests that boron affects the membrane structure and probably

the membrane components that are involved in phytochrome dependent shifts of membrane potentials. Borone has been found to stimulate the breaking of dormancy and germination of seeds (Metlitsky et al., 1972). They noticed that both the activity of β -glycosidase and the amount of phenolic inhibitor decrease in seeds with the breaking of dormancy. These workers also showed that the administration of boron, ether alone or in combination with gibberellic acid, resulted in an increased RNA content and an increase in α -amylase activity in germinating seeds (Creswell and Nelson ; 1973). Gibberellic acid alone produced similar but less pronounced. The positive effect of boron was not due to the biosynthesis of gibberellic acid as suggested by Creswell and Nelson (1973).

Nickel is widely distributed in plant kingdom (Malyuga, 1946) and has been considered as an essential nutrient for plants (Shkolnik, 1984). Nickel was found to stimulate α -amylase from barley (Corello and Boisio, 1955). The elevated activity of inorganic acid phosphatase in ageing detached rice leaves decreases when the leaves are kept in a solution containing 237.7 ppm. nickel (Mishra et al., 1973). Mishra and Kar (1974) reported that nickel control catalase activity in plants. According to Rubin and Chernavina (1970) nickel is associated with a dramatic increase in activities of ascorbate and phenol oxidases.

Table - 27

Effect of heavy metals (Cd, Pb, & Hg) on seed germination of S. viarum Dunal.

Treatment	Conc. (M)	% of germination	
		(60 days)	(270 days)
Cadmium chloride	10 ⁻²	65.5(2.091)**	0.0(-13.289) <
	10 ⁻³	70.6(3.931)*	18.2(9.315)*
	10 ⁻⁴	78.0(6.599)*	20.2(11.799)*
	10 ⁻⁵	71.0(4.075)*	18.4(9.553)*
	10 ⁻⁶	64.5(1.731)**	16.6(7.328)*
	10 ⁻⁷	62.2(0.902)	15.5(5.952)*
	Lead chloride	10 ⁻²	40.0(-7.104) <
10 ⁻³		58.0(-0.613)	0.0(-13.289) <
10 ⁻⁴		74.5(5.337)*	0.0(-13.289) <
10 ⁻⁵		68.4(3.137)*	10.0(-0.859)
10 ⁻⁶		65.4(2.055)**	18.5(9.688)*
10 ⁻⁷		62.0(0.829)	16.2(6.831)*
Mercuric chloride		10 ⁻²	12.2(-17.129) <
	10 ⁻³	35.4(-8.763) <	0.0(-13.289) <
	10 ⁻⁴	55.5(-1.514)	0.0(-13.289) <
	10 ⁻⁵	58.2(-0.541)	10.2(-0.621)
	10 ⁻⁶	70.0(3.714)*	12.5(2.235)**
	10 ⁻⁷	65.0(1.911)**	14.5(4.720)*
	Control	0±0	59.7 ± 6.2

* Significantly greater than control at 1% level of significance
 ** " " " " 5% " " "
 Significantly lesser than control at 1% level of significance
 " " " " 5% " " "

Though cadmium, lead and mercury have been regarded as pollutant and inhibitory to seed germination and seedling growth in a number of plants (Bazzaz et al., 1974; Mukherjee and Mukherjee, 1979) but the table-27 shows that they have the capacity to stimulate seed germination of S. viarum Dunal at certain level of concentration. Maximum stimulations of seed germination of 78%, 74.5% and 70% have been observed due to treatment of cadmium (10^{-4} M), lead (10^{-4} M) and mercury (10^{-6} M) respectively as compared to control (59.7%). The table-26 also shows that all these heavy metals have stimulated germination of deteriorated seeds of Solanum viarum Dunal specially at lower concentration. Maximum of 20.2% of seed germination has been observed due to treatment of cadmium at 10^{-4} M solution as compared to control. Thus S. viarum Dunal shows tolerance to the heavy metals. This may be due to uptake of metal into cell wall and vacuoles through an intensive carrier transporting system and evolution of metal resistant enzyme and alteration of cellular metabolism (Nissen and Bensen, 1982), and needs further investigation.

S U M M A R Y

Seed of Solanum viarum Dunal, stored for a longer period attains the state of non-viability and which has been experimentally demonstrated with tetrazolium test.

Seeds have been noted to attain non-viability after one year.

During invigouration treatment two types of seeds, one stored for 60 days and the other stored for 270 days have been taken into consideration.

Vitamins in general have been observed to stimulate seed germination and which has been observed to be statistically significant at certain concentrations over control. Thiamine and pyridoxine hydrochloride have been observed to show maximum stimulation of germination of seed stored for short and long time respectively.

Pyridoxine hydrochloride and pantothenic acid have been noted to become effective for the seeds stored for short and long time respectively when they are used in combination with IAA.

Out of different amino acids glutamic acid has been observed to show maximum stimulation of germination of seeds of both the types.

In connection with the effect of various sugars, though stimulation has been noticed in both types of seed significant stimulation is evident in case of shortly stored seeds.

Out of different growth regulating substance rutin and morin has been observed to show much stimulation in the seed stored for short duration. On the other hand morin is noted to show maximum stimulation in seeds stored for longer period.

Phenolic acids like resorcinol, salicylic acid and tannic acid have been observed to possess invigouration of seed at certain concentrations when used individually or in combination with gibberelic acid.

GA itself has marked invigouration capacity.

Phenoxy acetic acids have in general not stimulatory in function for both the types of seed, though much effect has been observed due to treatment of p-chloro phenoxy acetic acid specially on shortly storage seeds.

Dikegulac-sodium, maleic hydrazide, 8-azaguanine, and alar have also significant invigouration capacity.

The salt of heavy metals like cadmium, lead and mercury has also invigouration activity specially on shortly stored seed. Out of these chemicals the activity of cadmium

is maximum on seeds stored for longer period.

Though manganese , copper , cobalt , nickel and boron have invigouration activity, but the activity of manganese and copper have been shown to be significant statistically.