International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS™ | Volume 8 | Issue 4 | OCT-DEC | 2018 | 281-290
Research Article | Biological Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

INVIGOURATION OF SEEDS OF *Rauvolfia serpentina* and *Withania somnifera* BY CHEMICAL MANIPULATION TECHNIQUES

Amitava Nayek*

Department of Botany, Trivenidevi Bhalotia College, Raniganj, Dist. - Paschim Barddhaman – 713347, West Bengal, INDIA,

*Corresponding Author Email: amitavanayek@gmail.com

ABSTRACT

An experiment was conducted to arrest the ageing-induced fast loss of germination and also to enhance the permeability of seeds of two drug yielding plants viz. Rauvolfia serpentina (L.) Benth. (Apocynaceae) and Withenia somnifera Dunal. (Solanaceae) with antioxidants like reduced glutathione, mercaptoethanol, ascorbic acid, cysteine and a growth retardant salicylic acid. The seeds were pretreated with concentrations of 10^{-2} to 10^{-5} M for 8 Hr. The pretreated seeds were then imposed for accelerated ageing treatment for 20 and 40 days at 99.1% Relative Humidity (RH) at 30 ± 2 °C.

Field emergency capacity was found better in chemically pretreated seeds than non-treated seeds. Plant potentiality, measured in terms of chlorophyll and protein content, Hill activity was higher in the plants developed from pretreated seeds ignoring the adverse storage condition. Catalase and peroxidase enzyme activity was also remarkably supports Hill activity. Therefore, the trend of activities of antioxidants follows: reduced glutathione > salicylic acid > mercaptolethanol > ascorbic acid > cysteine. The most effective concentration was 10^{-4} M in both the cases antioxidant and growth retardant.

KEY WORDS

Rauvolfia serpentina, Withania somnifera, seed invigouration, chemical manipulation.

INTRODUCTION

Rauvolfia serpentina (L.) Benth. and *Withania somnifera* L.) Dunal. are the well known economically most important drug yielding plants grows in tropical and subtropical countries. Seed is the most important part of a plant. Good vigour of a seed is generally considered by its potentiality of timely uniform emergence and development of normal seedling in a wide range of field and weather conditions and even in suboptimal conditions. Moisture, Relative Humidity (RH) and temperature have a great influence on seed viability which ultimately affects the seed vigour. Yield of any crop depends upon the germination potential of seeds which determines seedling emergence and crop stand. Seed vigour gas has direct influence on the plant growth process involved in the production of yield (Vanangamudi *et al.,* 2006) [35]. In cultivation trials, propagation of *R. serpentina* is chiefly done through seeds. The rate of germination is quite variable ranging from 10 – 74% in case of fully matured heavy seeds (Hussain *et al*, 1993) [21]. Seedlings were raised in the nursery by direct sowing of the seeds in the field and have been found successful. So, for successful seedling development and high biomass production, the basic need is to raise seedling from healthy seed lots. Both coated and decoated seeds of *R. serpentina* showed no germination in absence of mycorrhizal association in normal as well as sterilize garden soil. Germination (80%) was found in sulfuric acid (18 N for 3 h) pretreated seeds with VAM association (Choudhury *et al.,* 2006) [13].

Storing of seeds is a serious problem in tropical or subtropical countries due to high temperature and relative humidity accelerate seed ageing phenomenon causing consequent deterioration and non-viability of seeds. The problem of retention of seed vigour in states of India is much more acute because of semi-arid climate and extremely high relative humidity prevailing during the major the part of a year which are very conducive to the growth of microorganisms, particularly fungi (Halder and Gupta, 1980) [18]. In fact, accelerated ageing treatment provides a powerful manipulative tool which makes it possible to study the process of seed deterioration over a very short period and this mimics the natural ageing process (Heydecker, 1972; Delouche and Baskin, 1973; Perl *et al*., 1978; Halder, 1981) [20,15,31,17].. Although efficacy of several classes of chemicals viz. hormones, retardants, redox chemicals, phenols, vitamins and salts on maintenance of seed health under storage has been established (Chhetri *et al*., 1993; Basu, 1994; Rai, 2000) [11,5,32], this field of seed physiology still remains relatively less explored. Thus, the major objective of this work is to test the efficacy of antioxidants like reduced glutathione, ascorbic acid, L-cysteine, ferrous sulfate and mercaptoethanol and a growth retardant, salicylic acid on seed vigour of *R. serpentina* an *W. somnifera* plants under storage.

MATERIALS AND METHODS:

SEED PRETREATMENT: Freshly collected seeds from reliable source of *Rauvolfia serpentina (L.) Benth.* (Apocynaceae) and *Withania somnifera (L.) Dunal.* (Solanaceae) were surface-sterilized by immersing in 0.1% $HgCl_2$ for 90 sec. and immediately rinsed with sterile distilled water. Seeds of *R. serpentina* were decoated with 18 N H_2SO_4 for 3 h followed by wash in running water for 2 h (Choudhury *et al*., 2006) [13]. Only decoated seeds of *R. serpentina* were used for further experiments. Seeds of this plants were separately presoaked in the graded solutions (10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} M) of reduced glutathione, ascorbic acid, L-cysteine, ferrous sulfate mercaptoethanol and salicylic acid or distilled water for 8 h. Then the seeds were dried back to the original dry weight by intense sun drying followed by measuring its original moisture level. The pretreated seed lots (300 g each) were taken in separate porous cloth bags for each treatment and thus stored in separate desiccators. The desiccators were preimposed with 300 ml 3.03% H_2SO_4 to maintain the RH at 99.1% (Rao *et al.,* 2003) [33]. This experimental set-up was kept at 30 ± 2 °C at dark for 40 days allowing the seeds to experience forced ageing treatment. The concentration of H_2SO_4 was checked and changed at 7 days intervals to maintain the desired RH within the desiccators. The treated seeds of *R. serpentina* were soaked in distilled water at 30 ± 2 °C in the dark for a full day. After imbibition, seeds were taken out and inoculated with VAM fungi. Then the VAM fungi inoculated seeds were transferred to small earthen pots containing normal and sterilized garden soil. The whole set up then kept at 30 ± 2 °C in 16 h light and 8 h dark photoperiod and allowed to germinate (Choudhury *et al*., 2006) [13].

EXPERIMENTAL PROCEDURE: Germination, field emergence capacity of seeds was made after 0, 20 and 40 days of accelerated ageing treatment. Germination counts were performed by recording number of seeds with 2 mm of radical emergence through the testa. Field emergence capacity was recorded after 14 days of seed sowing in the experimental field. Viability test was done by TTC method. Seeds were placed in 0.1% TTC (2,3,5-triphenyl tetrazolium chloride) solution at 37 °C for 3 h. Reddish-pink color of formazen in the embryonic axis denoted viability (ISTA, 1976) [23]. Tissue permeability and leakage of ions was determined by immersed the seeds (300 g) in 30 ml deionized water for 6 h. The ionic concentration of the bathing medium of the respective tissues was recorded through a direct reading conductivity meter. Extraction and estimation of chlorophyll was done by following the method proposed by Arnon (1949) [3]. For measurement of Hill activity, chloroplasts were prepared by gentle homogenization of leaves (300 mg) in a mortar at 0 to 4 °C in isolation medium containing 50 mM sodium phosphate buffer (pH 7.5), 50 mM NaCl, 3 mM $MgCl_2$ and 0.5% (w/v) BSA. Mercaptoethanol at 5 mM was also included in the medium used for isolating chloroplasts but was omitted from the determination medium. The Hill activity of isolated chloroplasts was determined by the photoreduction of 2,6-dichloroindophenol (DCIP) measured at 620 nm in a Spectrophotometer following a 10 min illumination at 20 °C and quantum flux density 150 µmol $m^{-1}s^{-1}$. The determination medium (10 ml) contained: 45 mM sodium phosphate buffer (pH 7.5), 45 mM NaCl, 3 mM $MgCl_2$, 0.012 mM DCIP, 0.045% (w/v) BSA and about 20 µg chlorophyll.

Catalase activity of was analysed by following the method of Snell and Snell (1971) [34] and modified by

Biswas and Choudhuri (1978) [10]. The activity of peroxidase was analysed as per the method of Kar and Mishra (1976) [25]. In the enzyme assay, values at zero time were taken as blanks and the activity of each enzyme was expressed as [(A X Tv / (t X v)], where A is the absorbance of the sample after incubation minus the absorbance at zero time (min) of incubation with substrate and the v is the volume of the filtrate taken for incubation (Fick and Qualset, 1975)[16].

Each treatment was replicated three times and then means value are given in the tables. The data were statistically analyzed at the treatment and replication levels and LSD values at the 5% level are included in the tables.

RESULTS AND DISCUSSION:

The investigation shows the storage potential enhancement property of the test chemicals *viz*. antioxidant reduced glutathione, mercaptoethanol, ascorbic acid, cysteine and growth retardant salicylic acid. Cessation of loss of germination (Table-I) and field emergence capacity (Table-III) as well as increment of permeability (Table-II) was recorded and which indicates the activity of the chemicals. Contents of Chlorophyll, Protein and Hill activity were found higher in pretreated seed samples in 0 day accelerated ageing seeds (Table-IV). The contents of Chlorophyll, Protein and Hill activity were much higher in 20 days accelerated ageing seeds (Table-V). These experiments were not carried out in 40 days accelerated ageing seeds due to less filed emergence capacity. Catalase (Table-VI) (Abdul Baki and Anderson, 1972; Yadav *et al*., 2003) [1,36] and peroxidase (Table-VII) (Bhattacharjee and Choudhuri, 1986; Yadav *et al*., 2003) [8,36] activities are generally used as very reliable indices for the evaluation of seed viability. In fact, these two are considered as potential scavenger enzymes which can efficiently detoxify harmful metabolite like H_2O_2 and thus help alleviating undesired toxic environment at the cellular level. Hence higher activities of CAT, POD and superoxide dismutase etc. are reported as strong defense elements in plant system (Yadav *et al*., 2003; Kar and Mishra, 1976; Pati, 2011; Pati and Bhattacharjee, 2011) [36,25,28,29]. High level of CAT activity in high vigour seeds have also been reported (Bhattacharjee 1984; Bhattacharjee *et al*., 1999) [6,9]. Ageing-induced loss of germination and arrested increase of permeability as well as enhanced field emergence capacity are considered to be the important visible criteria for the evaluation of poor seed vigour (Anderson, 1970; Halder *et al*., 1983; Rai, 2000) [2,19,32]. Content of chlorophyll and protein, Hill activity, activities of catalase and peroxidase are considered as the reliable indicators of vigour status of plants. In this investigation, comparatively better healthy plant and plants with higher metabolic status was raised from the seeds pretreated with test chemicals even in under adverse storage condition. This may be considered as the indicative of invigoration of seeds under storage. Similarly, invigorated seeds exhibit better field performance which is measured in terms of plant growth and metabolism. Superior performance of plants raised from high vigour seeds (Rai, 2000) [32]. Plant growth and metabolism were found comparatively better in pretreated seeds. Results indicate the hardening or invigoration property of the pretreating agents. Such hardening effect on seed was reflected in plant growth and metabolism.

In this study, the cessation of increase of permeability of seeds clearly indicates that the membrane integrity of tissue of seeds is maintained by the test chemicals. Reduced glutathione, a redox buffer and reducing agent, removes toxic peroxides formed in normal course of growth by the activity of glutathione peroxidase containing a covalently bond selenium atom. Seed germination and seedling development are regulated solely by phytochrome which promotes seed germination. The P_{fr} influences gibberellins synthesis and alters membrane permeability. The activities of storage product enzymes α-amylase, phosphate and ribonuclease are mediated by gibberellins (Copeland and Mc Donald, 2001) [14]. The chemicals used in this study may influence on the role of phytochrome localized in cell membrane. In conclusion, the effects were in general reduced glutathione > salicylic acid > mercaptoethanol >ascorbic acid > cysteine. The chemical-pretreated seeds retained higher seed vigour and produced healthier plants. Thus, the chemicals tested may be considered as potential seed invigoration agents.

CONCLUSION: The major findings of this study were that the ageing induced loss of germination of the seeds of two drug yielding plants might be overcome by chemical manipulation treatment. The field emergence capacity and field performance would be better in the treated seeds than that of the untreated ones. So, the productivity might be higher if pretreated with stipulated chemical(s). The most effective concentration of the antioxidants and salicylic acid were 10^{-4} M and the most effective antioxidant was reduced glutathione and the least effective one cysteine as recorded. The observations found similar in both the plants *Rauvolfia serpentina* and *Withania somnifera.*

Table-I: Effect of seed pretreatment with graded solutions of antioxidants and salicylic acid (10^{-2} - 10^{-5} M) on percentage germination of *Rauvolfia serpentina* and *Withania somnifera* viable seeds stored under accelerated ageing condition for 0, 20 and 40 days at 99.1% RH and 30 ± 2 °C.

Treatment	Concentration (M)	Percentage germination Species and accelerated ageing days					
		Rauvolfia			*Withenia*		
		0	20	40	0	20	40
Control	10^{-2}	80	38	20	97	63	31
	10^{-3}	81	39	20	98	65	31
	10^{-4}	82	45	23	98	66	35
	10^{-5}	80	36	19	97	63	32
Reduced glutathione	10^{-2}	80	43	22	97	58	31
	10^{-3}	80	44	25	98	59	33
	10^{-4}	81	52	33	98	64	41
	10^{-5}	79	46	25	97	60	35
Mercaptoehanol	10^{-2}	81	43	22	97	58	33
	10^{-3}	81	44	24	98	57	34
	10^{-4}	80	49	29	99	62	39
	10^{-5}	79	45	23	98	58	35
Ascorbic acid	10^{-2}	81	41	23	97	56	32
	10^{-3}	81	42	25	97	56	31
	10^{-4}	82	46	28	98	58	36
	10^{-5}	79	42	24	98	56	32
Cysteine	10^{-2}	82	41	21	97	51	29
	10^{-3}	81	43	22	98	54	28
	10^{-4}	81	45	26	99	56	30
	10^{-5}	80	41	23	98	54	29
Salicylic acid	10^{-2}	81	43	24	96	57	36
	10^{-3}	81	44	25	97	58	34
	10^{-4}	80	52	30	98	62	40
	10^{-5}	80	46	26	97	59	37
LSD at P=0.05	N. A	0.001	1.23	1.55	0.002	1.67	1.73

TABLE-II: Effect of seed pretreatment with graded solutions of antioxidants and salicylic acid (10^{-2} - 10^{-5} M) on permeability of *Rauvolfia serpentina* and *Withania somnifera* viable seeds stored under accelerated ageing condition for 0, 20 and 40 days at 99.1% RH and 30 ± 2 °C.

Treatment	Concentration (M)	Permiability (µmho g^{-1} fresh wt) Species and accelerated ageing (days)					
		Rauvolfia			*Withenia*		
		0	20	40	0	20	40
Control	10^{-2}	314.3	343.2	428.6	326.1	363.4	434.8
	10^{-3}	316.1	346.1	430.1	327.7	365.1	437.2
	10^{-4}	317.5	348.7	433.8	329.8	366.6	439.7
	10^{-5}	316.6	345.4	432.2	328.9	364.5	438.4
Reduced glutathione	10^{-2}	317.1	322.4	324.7	331.8	339.6	348.1
	10^{-3}	318.3	323.7	326.6	333.5	340.5	349.6
	10^{-4}	319.6	325.2	327.6	334.1	342.1	351.5
	10^{-5}	318.4	324.1	326.2	332.2	341.4	350.2
Mercaptoehanol	10^{-2}	313.2	323.6	331.7	328.9	339.4	345.1
	10^{-3}	314.7	325.1	332.8	330.1	341.6	346.6
	10^{-4}	316.4	326.3	334.2	331.9	342.7	348.7
	10^{-5}	315.8	327.3	332.2	330.2	341.5	347.1
Ascorbic acid	10^{-2}	315.2	321.1	325.2	327.2	336.4	340.2
	10^{-3}	316.4	322.7	326.1	328.1	338.1	342.1
	10^{-4}	317.3	324.4	328.4	330.8	341.1	343.6
	10^{-5}	316.1	323.6	327.4	329.0	339.5	342.0
Cysteine	10^{-2}	314.8	330.7	336.4	326.3	344.7	450.6
	10^{-3}	315.6	332.4	338.1	328.1	346.6	452.2
	10^{-4}	318.8	336.1	341.2	331.8	348.5	453.8
	10^{-5}	316.1	334.3	339.6	330.5	345.2	451.1
Salicylic acid	10^{-2}	316.4	321.7	329.1	325.1	332.1	336.3
	10^{-3}	317.5	323.2	330.5	326.4	334.6	338.1
	10^{-4}	320.6	324.4	334.1	329.5	338.2	341.4
	10^{-5}	318.1	321.5	332.7	327.4	336.3	338.5
LSD at P=0.05		0.004	1.03	1.44	0.003	1.71	1.28

TABLE -III: Effect of seed pretreatment with graded solutions of antioxidants and salicylic acid (10^{-2} - 10^{-5} M) on field emergence capacity of *Rauvolfia serpentina* and *Withania somnifera* viable seeds stored under accelerated ageing condition for 0, 20 and 40 days at 99.1% RH and 30 ± 2 °C.

Treatment	Concentration (M)	Field emergence capacity (%) Species and accelerated ageing (days)					
		Rauvolfia			*Withenia*		
		0	20	40	0	20	40
Control	10^{-2}	83.4	37.8	13.2	86.3	44.8	17.4
	10^{-3}	84.6	39.3	14.5	87.4	45.6	18.9
	10^{-4}	85.1	40.5	15.6	88.6	46.2	20.7
	10^{-5}	83.2	38.1	14.8	87.5	44.5	19.1
Reduced glutathione	10^{-2}	83.1	44.5	20.4	86.4	52.7	26.4
	10^{-3}	84.2	45.7	21.5	85.2	53.1	27.5
	10^{-4}	85.1	46.6	22.4	88.6	54.3	28.1
	10^{-5}	84.8	45.1	21.4	87.0	53.1	27.8
	10^{-2}	83.4	43.1	19.8	85.8	47.8	22.4

Treatment	Concentration (M)	Field emergence capacity (%) Species and accelerated ageing (days)					
		Rauvolfia			***Withenia***		
		0	20	40	0	20	40
Mercaptoehanol	10^{-3}	84.6	44.5	20.4	87.4	48.7	23.7
	10^{-4}	85.1	45.2	21.6	88.6	50.2	24.5
	10^{-5}	84.7	44.2	20.4	87.7	48.1	23.6
	10^{-2}	83.5	42.3	19.8	85.5	46.5	20.5
Ascorbic acid	10^{-3}	84.5	44.5	20.4	86.8	47.3	21.3
	10^{-4}	85.1	45.3	22.1	88.6	49.9	22.8
	10^{-5}	84.8	43.1	21.0	87.7	47.2	20.1
	10^{-2}	83.5	39.5	16.8	85.8	45.8	19.5
Cysteine	10^{-3}	84.6	41.4	17.6	87.2	46.4	20.4
	10^{-4}	85.1	42.5	18.0	88.6	47.7	21.9
	10^{-5}	84.9	41.1	17.2	87.1	46.5	19.1
	10^{-2}	83.4	43.7	18.8	86.4	49.0	23.7
Salicylic acid	10^{-3}	84.5	44.6	19.7	87.4	50.4	24.6
	10^{-4}	85.1	45.1	20.9	88.6	51.4	25.5
	10^{-5}	84.5	43.7	19.4	86.4	50.3	23.7
LSD at P=0.05		0.002	1.17	0.93	0.003	2.11	0.62

TABLE-IV:_Effect of seed pretreatment with graded solutions of antioxidants and salicylic acid (10^{-2} - 10^{-5} M) followed by accelerated ageing treatment for 0 day at 99.1% RH and 30 ± 2 °C on changes in chlorophyll, protein (mg g^{-1} fr wt.) and Hill activity (µmol 2,6-dichloroindophenol reduced mg^{-1} chlorophyll h^{-1}) of leaves of 30 days old plants.

Treatment	**Concentration (M)**	***Rauvolfia***			***Withenia***		
		Chlorophyll	**Protein**	**Hill activity**	**Chlorophyll**	**Protein**	**Hill activity**
	10^{-2}	2.68	30.4	211.8	2.42	35.1	286.7
Control	10^{-3}	2.71	31.7	213.2	2.43	36.4	289.3
	10^{-4}	3.01	34.5	217.5	2.49	38.8	298.7
	10^{-5}	2.82	32.2	214.6	2.43	36.6	294.6
	10^{-2}	3.76	40.6	309.8	4.02	47.9	285.4
Reduced	10^{-3}	3.79	41.2	311.1	4.09	48.2	286.3
glutathione	10^{-4}	3.97	44.6	315.4	4.41	54.7	295.4
	10^{-5}	3.86	43.8	314.2	4.20	53.2	292.7
	10^{-2}	3.35	36.4	281.6	4.16	43.7	265.4
Mercaptoehanol	10^{-3}	3.38	37.3	283.1	4.18	44.3	266.2
	10^{-4}	3.53	41.3	292.6	4.43	47.1	273.9
	10^{-5}	3.48	40.1	288.4	4.30	46.8	269.8
	10^{-2}	3.33	34.2	293.4	3.87	36.1	271.4
Ascorbic acid	10^{-3}	3.36	35.7	295.7	3.94	37.4	273.5
	10^{-4}	3.60	39.4	297.4	4.47	43.5	295.6
	10^{-5}	3.52	38.1	294.4	4.25	41.6	278.9
	10^{-2}	3.42	31.8	208.9	2.34	36.8	284.7
Cysteine	10^{-3}	3.48	33.7	210.4	2.46	37.5	286.3
	10^{-4}	3.76	37.1	218.7	2.86	44.4	295.4
	10^{-5}	3.66	36.3	215.4	2.68	42.6	291.4
	10^{-2}	3.45	33.8	261.4	4.01	36.4	263.5
Salicylic acid	10^{-3}	3.49	35.4	263.2	4.08	37.3	265.2
	10^{-4}	3.87	41.6	268.1	4.12	38.7	268.4
	10^{-5}	3.67	39.4	263.7	4.02	36.2	262.0
LSD at P=0.05		0.002	0.003	0.011	0.004	0.014	0.026

TABLE-V: Effect of seed pretreatment with graded solutions of antioxidants and salicylic acid (10^{-2} - 10^{-5} M)) followed by accelerated ageing treatment for 20 days at 99.1% RH and 30 ± 2 °C on changes in chlorophyll, protein (mg g^{-1} fr wt.) and Hill activity (µmol 2,6-dichloroindophenol reduced mg^{-1} chlorophyll h^{-1}) of leaves of 30 days old plants.

Treatment	Concentration (M)	*Rauvolfia*			*Withenia*		
		Chlorophyll	Protein	Hill activity	Chlorophyll	Protein	Hill activity
	10^{-2}	1.57	19.5	179.5	1.62	24.3	183.8
Control	10^{-3}	1.58	20.4	181.6	1.65	25.7	187.4
	10^{-4}	1.61	23.7	193.4	1.93	29.3	194.1
	10^{-5}	1.54	22.5	189.3	1.78	28.9	192.7
	10^{-2}	2.14	26.5	213.6	2.25	30.3	221.6
Reduced	10^{-3}	2.17	27.0	215.7	2.27	31.2	225.4
glutathione	10^{-4}	2.61	32.1	231.6	2.45	38.7	231.2
	10^{-5}	2.41	30.4	221.4	2.36	36.1	229.7
	10^{-2}	1.94	24.1	207.8	2.04	29.5	215.8
Mercaptoehanol	10^{-3}	1.97	25.3	209.1	2.08	30.4	217.4
	10^{-4}	2.21	29.4	217.5	2.31	34.0	224.1
	10^{-5}	2.10	28.7	214.6	2.17	33.2	221.0
	10^{-2}	1.88	23.7	205.4	1.86	25.3	214.5
Ascorbic acid	10^{-3}	1.91	24.2	206.3	1.97	27.4	216.0
	10^{-4}	2.13	28.4	213.5	2.25	34.0	232.1
	10^{-5}	2.01	27.1	209.1	2.10	32.5	221.3
	10^{-2}	1.66	25.4	185.2	1.69	24.8	195.6
Cysteine	10^{-3}	1.68	21.7	186.4	1.73	26.7	197.4
	10^{-4}	1.91	25.8	193.2	1.86	32.3	208.2
	10^{-5}	1.76	23.9	190.1	1.79	30.2	203.1
	10^{-2}	2.01	24.8	208.9	2.14	28.7	218.7
Salicylic acid	10^{-3}	2.07	26.1	211.0	2.17	30.6	219.9
	10^{-4}	2.26	32.2	221.1	2.41	36.3	227.8
	10^{-5}	2.18	29.4	215.4	2.28	33.2	223.4
LSD at P=0.05		0.005	0.003	0.011	0.008	0.014	0.026

TABLE-VI: Effect of seed pretreatment with graded solutions of antioxidants and salicylic acid (10^{-2} - 10^{-5} M) followed by accelerated ageing treatment for 0 and 20 days at 99.1% RH and 30 ± 2 °C on changes in catalse activity (unit/h/gfr.wt.) of leaves of 30 days old plants.

Treatment	Concentration (M)	Catalse activity (unit/h/gfr.wt.) Species and accelerated ageing (days)			
		Rauvolfia		*Withenia*	
		0	20	0	20
	10^{-2}	101.1	69.3	96.8	71.8
Control	10^{-3}	105.6	71.2	97.6	73.2
	10^{-4}	112.4	77.1	99.4	75.1
	10^{-5}	109.2	74.3	98.4	73.4
	10^{-2}	102.5	73.4	104.8	74.5
Reduced glutathione	10^{-3}	104.7	74.5	108.3	76.1
	10^{-4}	111.7	76.1	113.2	78.5
	10^{-5}	107.6	74.8	109.4	76.4
	10^{-2}	103.2	77.4	104.5	75.2
Mercaptoehanol	10^{-3}	105.3	78.5	105.4	77.1
	10^{-4}	110.4	80.1	109.3	79.6

Treatment	Concentration (M)	Catalse activity (unit/h/gfr.wt.) Species and accelerated ageing (days)			
		Rauvolfia		*Withenia*	
		0	20	0	20
	10^{-5}	108.6	76.2	107.1	76.4
Ascorbic acid	10^{-2}	99.4	73.5	103.4	73.1
	10^{-3}	101.5	74.6	104.5	76.3
	10^{-4}	107.6	78.1	107.3	79.5
	10^{-5}	104.2	74.2	105.1	74.9
Cysteine	10^{-2}	98.6	79.4	100.3	76.4
	10^{-3}	99.1	80.2	101.5	77.5
	10^{-4}	106.5	83.6	104.3	79.9
	10^{-5}	103.4	81.0	102.6	77.2
Salicylic acid	10^{-2}	100.0	69.4	100.5	75.9
	10^{-3}	102.2	70.6	102.1	76.1
	10^{-4}	104.1	75.3	106.2	78.4
	10^{-5}	103.2	72.5	102.4	75.5
LSD at P=0.05		2.003	1.05	2.006	1.04

TABLE-VII: Effect of seed pretreatment with graded solutions of antioxidants and salicylic acid (10^{-2} - 10^{-5} M) followed by accelerated ageing treatment for 0 and 20 days at 99.1% RH and 30 ± 2 °C on changes in peroxidase activity (unit/h/gfr.wt.) of leaves of 30 days old plants.

Treatment	Concentration (M)	Peroxidase activity (unit/h/gfr.wt.) Species and accelerated ageing (days)			
		Rauvolfia		*Withenia*	
		0	20	0	20
	10^{-2}	95.3	59.7	96.3	56.4
	10^{-3}	97.5	61.2	97.8	58.9
Control	10^{-4}	100.2	65.1	101.5	62.4
	10^{-5}	98.1	62.1	98.2	59.1
	10^{-2}	94.3	55.6	96.4	55.4
	10^{-3}	96.1	57.1	97.6	57.1
Reduced glutathione	10^{-4}	99.5	59.8	99.8	61.2
	10^{-5}	94.1	56.4	95.4	56.7
	10^{-2}	96.5	56.7	95.3	54.6
	10^{-3}	98.4	58.9	97.5	57.3
Mercaptoehanol	10^{-4}	102.3	63.4	103.4	63.1
	10^{-5}	99.1	61.1	96.2	58.4
	10^{-2}	96.5	57.6	96.7	56.7
	10^{-3}	98.2	59.8	98.5	57.4
Ascorbic acid	10^{-4}	104.1	64.2	103.4	64.2
	10^{-5}	97.5	61.1	97.6	61.0
	10^{-2}	97.8	56.3	98.1	55.9
	10^{-3}	98.9	57.4	99.8	57.4
Cysteine	10^{-4}	105.1	63.4	105.1	64.3
	10^{-5}	101.2	58.2	101.4	60.0
	10^{-2}	95.8	57.6	96.7	57.0
	10^{-3}	97.4	59.4	98.4	58.9
Salicylic acid	10^{-4}	101.3	64.1	104.6	63.1
	10^{-5}	97.5	60.4	99.1	60.1
LSD at P=0.05		2.006	1.04	2.005	1.06

ACKNOWLEDGEMENT:

The author is thankful to Triveni Devi Bhalotia College, Raniganj for providing the necessary infrastructure facilities and funding required for the present study. The author also expresses his sincere thanks and gratitude to the co-workers and staff of the Department of Botany of the College for their constant support and co-operation. The author expresses his sincere thanks to Prof. Aloke Bhattacharjee, Senior Professor of Botany (Retired), Burdwan University for his constant support, encouragement, guidance.

REFERENCES

[1] Abdul-Baki A.A and Anderson J.D, Physiological and biochemical determination of seeds. In Seed Biology, Vol. II, ed. T. Kozowski, Academic Press, New York, pp. 203-215. (1972).

[2] Andderson, J.D.,Metabolic changes in partially dormant wheat seeds during storage. Plant Physiol. 46: 605-608. (1970).

[3] Arnon D.I, Copper enzymes in isolated chloroplasts. Polyphenol oxidase in Beta vulgaris. Plant Physiol., 24: 1-15. (1949).

[4] Association of Official Seed Analysts. Rules for Testing Seeds. Proceedings of the Association of Official Seed Analysts. 60 (2) 39. (2000).

[5] Basu R. N., An appraisal of research on wet and dry physiological seed treatments and their applicability with special reference to tropical and subtropical countries. Seed Science and Technology, 22, 107-126. (1994).

[6] Bhattacharjee A., Responses of sunflower plants towards growth retardants with special reference to growth, metabolism and yield. Ph.D. Thesis, Burdwan University, India. (1984).

[7] Bhattacharyya A.K. and Basu R.N., Retention of vigor and viability of stored pea (Pisum sativum L.) seed. Indian Agriculturarist. 34: 187-193. (1990).

[8] Bhattacharjee A. and Choudhuri M.A., Chemical manipulation of seed longevity and stress tolerance capacity of seedlings of Corchorus capsularis and C. olitorius. J. Plant Physiol., 125: 391-400. (1986).

[9] Bhattacharjee A., Chattopadhyay A., Rai A.S., Potentiation of safflower seeds under artificial stress storage environment by chemical growth retardants. Seed Sci. Technol., 27: 707-719. (1999).

[10] Biswas A.K. and Choudhuri M.A., Differential behaviour of the flag leaf of intact rice plant during ageing. Biochem. Physiol. Pflanzen. 173: 220-228. (1978).

[11] Chhetri et al. Seed Science and Technology. 21: 31-44. (1993).

[12] Chhetri D.R., Rai A.S., Bhattacharjee A., Chemical manipulation of seed longevity of four crop species in an unfavourable storage environment. Seed Sci. Technol., 21: 31-44. (1993).

[13] Choudhury et al., Mycorrhizas in the perennial grasses of Cholistan desert, Pakistan. Intl. J. Bot. 2: 210-218. (2006).

[14] Copland L.O., Mc Donald M.B., In Principles of Seed Sc. and Technol. (4th edn.), Norwell, Massachusetts: Kluwer Academic Publishers, USA. (2001).

[15] Delouche J.C., Baskin C.C., Accelerated aging techniques for predicting the relative storability of seed lots. Seed Science and Technology 1: 27-452. (1973).

[16] Fick N.G. and Qualset C.O., Genetic control of endosperm amylase activity and gibberellin responses in standard height and short statured wheat. Proceedings of National Academy of Science. USA. 72: 892-895. (1975).

[17] Halder S., Studies on viability, yield and associated biochemical changes in leaves during seed filling in sunflower (Helianthus annuus L. cv. EC 68414). Ph.D. Thesis, Burdwan University, India. (1981).

[18] Halder S. and Gupta K., Effects of storage of Sunflower seeds in high and low relative humidity on solute leaching and internal biochemical changes. Seed Science and Technology. 8: 317-321. (1980).

[19] Halder S., Koley S., Gupta K.,On the mechanism of sunflower seed deterioration under two different types of accelerated ageing. Seed Sci. Technol., 11: 331-339. (1983).

[20] Heydecker W., Vigour. In E.H. Roberts (ed.), Viability of seeds (pp. 209-252). London: Chapman and Hall. (1972).

[21] Hussain et al., Germination promotion of Ziziphus nummularia. Hamdard. 36, 46-55. (1993).

[22] International Seed Testing Association [ISTA]. Seed Vigour Testing. International Rules for Seed Testing, Zurich, Switzerland. (2014).

[23] International Seed Testing Association, ISTA, International rules for seed testing. Seed Sci. Technol., 4: 51-177. (1976).

[24] Hampton J.G. and Te Korny D. M. (eds.), Handbook of vigour test methods (Third edition). Zurich: International Seed Testing Association. (1995).

[25] Kar M, Mishra D., Catalase, peroxidase, polyphenol oxidase activities during rice leaf senescence. Plant Physiol., 57: 315-319. (1976).

[26] Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J., Protein measurement with the folin-phenol reagent. J. Biol. Chem., 193: 265275. (1951).

[27] Mishra V.K., Studies on seed vigour and viability of a few pulse crops and devising techniques of seed invigouration. Ph.D. Thesis, Vidyasagar University, India. (2006).

[28] Pati C.K., Seed invigouration, plant potentiation and yield augmentation of two promising pulse crops (Lathurus sativus L. and Vinna mungo (L.) Hepper) by chemical manipulation. Ph.D. thesis,Vidyasagar University, West Bengal, India. (2007).

[29] Pati C.K. and Bhattacharjee A.,Sunflower seed invigoration by chemical manipulation. African Journal of Plant Science. Vol. 5(15), pp. 867-872. (2011).

[30] Pati C.K., Bhattacharjee A., Alleviation of seed deterioration and enhancement of plant potential of a pea species using selected medicinal plant extracts. J. Med. Plant Res., 5(17): 4108-4111. (2011).

[31] Perl M., Luria I., Gelmond H., Biochemical changes in sorghum seeds affected by accelerated ageing. J. Expt. Bot. 29: 497-509. (1978).

[32] Rai A.S., An investigation into the problems of maintenance of seed vigour and viability under adverse climatic condition of Darjeeling hills. Ph.D. Thesis. North Bengal University, West Bengal, India. (2000).

Received:07.08.18, Accepted: 08.09.18, Published:01.10.2018

[33] Rao R.G.S., Singh P.M., Singh K.P., Deterioration of onion seeds under accelerated ageing conditions. Indian J. Plant Physiol. Special Issue: 189-194. (2003).

[34] Snell F.D., Snell C.T., Colorimetric Methods of Analysis. Van Nostrand Reinhold Co., New York, IV AAA, pp. 7-145. (1971).

[35] Vanangamudi, V. et. al.,Seed Hardening, Pelleting, and Coating Principles and Practices, Satish Serial Publishing House, ISBN: 9788189304201. (2006).

[36] Yadav S., Bhatia V.S., Guruprasad K.N., Role of peroxidase and catalase enzymes in deterioration of soyabean seeds due to field weathering. Indian J. Plant Physiol., Special Issue, pp. 195-200. (2003).

***Corresponding Author:**
Amitava Nayek*
Email: **amitavanayek@gmail.com**