



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

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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 *Withania 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 > mercaptoethanol > 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 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* and *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% HgCl₂ for 90 sec. and immediately rinsed with sterile distilled water. Seeds of *R. serpentina* were decoated with 18 N H₂SO₄ 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% H₂SO₄ 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 H₂SO₄ 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 formazan 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 MgCl₂ 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⁻²s⁻¹. The determination medium (10 ml) contained: 45 mM sodium phosphate buffer (pH 7.5), 45 mM NaCl, 3 mM MgCl₂, 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 $[(A \times T_v) / (t \times V)]$, 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 field 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					
		<i>Rauvolfia</i>			<i>Withania</i>		
		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
Mercaptoethanol	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 ($\mu\text{mho g}^{-1}$ fresh wt)					
		Species and accelerated ageing (days)					
		<i>Rauvolfia</i>			<i>Withenia</i>		
		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)					
		<i>Rauvolfia</i>			<i>Withenia</i>		
		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
Mercaptoethanol	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
Control	10 ⁻²	2.68	30.4	211.8	2.42	35.1	286.7
	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
Reduced glutathione	10 ⁻²	3.76	40.6	309.8	4.02	47.9	285.4
	10 ⁻³	3.79	41.2	311.1	4.09	48.2	286.3
	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
Mercaptoethanol	10 ⁻²	3.35	36.4	281.6	4.16	43.7	265.4
	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
Ascorbic acid	10 ⁻²	3.33	34.2	293.4	3.87	36.1	271.4
	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
Cysteine	10 ⁻²	3.42	31.8	208.9	2.34	36.8	284.7
	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
Salicylic acid	10 ⁻²	3.45	33.8	261.4	4.01	36.4	263.5
	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
Control	10^{-2}	1.57	19.5	179.5	1.62	24.3	183.8
	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
Reduced glutathione	10^{-2}	2.14	26.5	213.6	2.25	30.3	221.6
	10^{-3}	2.17	27.0	215.7	2.27	31.2	225.4
	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
Mercaptoethanol	10^{-2}	1.94	24.1	207.8	2.04	29.5	215.8
	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
Ascorbic acid	10^{-2}	1.88	23.7	205.4	1.86	25.3	214.5
	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
Cysteine	10^{-2}	1.66	25.4	185.2	1.69	24.8	195.6
	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
Salicylic acid	10^{-2}	2.01	24.8	208.9	2.14	28.7	218.7
	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 catalase activity (unit/h/gfr.wt.) of leaves of 30 days old plants.

Treatment	Concentration (M)	Catalase activity (unit/h/gfr.wt.)			
		Species and accelerated ageing (days)			
		Rauvolfia		Withenia	
		0	20	0	20
Control	10^{-2}	101.1	69.3	96.8	71.8
	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
Reduced glutathione	10^{-2}	102.5	73.4	104.8	74.5
	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
Mercaptoethanol	10^{-2}	103.2	77.4	104.5	75.2
	10^{-3}	105.3	78.5	105.4	77.1
	10^{-4}	110.4	80.1	109.3	79.6

Treatment	Concentration (M)	Catalase activity (unit/h/gfr.wt.) Species and accelerated ageing (days)			
		<i>Rauvolfia</i>		<i>Withenia</i>	
		0	20	0	20
Ascorbic acid	10 ⁻⁵	108.6	76.2	107.1	76.4
	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)			
		<i>Rauvolfia</i>		<i>Withenia</i>	
		0	20	0	20
Control	10 ⁻²	95.3	59.7	96.3	56.4
	10 ⁻³	97.5	61.2	97.8	58.9
	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
Reduced glutathione	10 ⁻³	96.1	57.1	97.6	57.1
	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
Mercaptoethanol	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
	10 ⁻⁴	104.1	64.2	103.4	64.2
Ascorbic acid	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
	10 ⁻⁴	105.1	63.4	105.1	64.3
	10 ⁻⁵	101.2	58.2	101.4	60.0
Cysteine	10 ⁻²	95.8	57.6	96.7	57.0
	10 ⁻³	97.4	59.4	98.4	58.9
	10 ⁻⁴	101.3	64.1	104.6	63.1
	10 ⁻⁵	97.5	60.4	99.1	60.1
	10 ⁻²	95.8	57.6	96.7	57.0
LSD at P=0.05		2.006	1.04	2.005	1.06

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