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EFFECT OF GROWTH REGULATORY HORMONES ON THE GERMINATION OF STORED SEEDS OF *WITHANIA SOMNIFERA* (WS-100), *BOERHAAVIA DIFFUSA* (VAR. LOCAL) AND *PHYLLANTHUS FRATERNUS* (VAR. LOCAL)

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ABSTRACT:

In the present study the effect of gibberellic acid (GA₃) and kinetin (KIN) on the germination and dormancy on the stored seeds of medical plant, *Withania somnifera* (Var. WS 100), *Boerhaavia diffusa* (Var. Local) and *Phyllanthus fraternus* (Var. Local) had been studied. Seeds were stored at constant temperature in desiccators and the germination data was taken up to 2 years with interval of 6 months. The parameters include are speed of germination (QI), average root length (SVI) and average total length (SVI – II). In addition to these parameters, the measurements of average root length, average shoot length, average total length, average fresh weight, and average dry weight, average root length, average shoot length, average total length, average fresh weight, average dry weight were also calculated. The quantity/concentration of both plant growth regulators was used between 10⁻⁵M to 10⁻⁷ M. The presoaking treatment was given to seeds. The findings of the study show that gibberellic acid (GA₃) with 10⁻⁵ M and kinetin (KIN) 10⁻⁷ M are better for acquiring % germination (%G), the quality index and seedling vigour index (SVI-1 and SVI-11) in fresh and partially aged (1 year and 2 year) seeds of *Withania somnifera*, gibberellic acid (GA₃) with 10⁻⁶ M and kinetin (KIN) 10⁻⁶ M are better for acquiring % germination (%G), the quality index and seedling vigour index (SVI-1 and SVI-11) in fresh and partially aged (1 year and 2 year) seeds of

Boerhaavia diffusa and gibberellic acid (GA₃) with 10⁻⁶ M and kinetin (KIN) 10⁻⁶ M are better for acquiring % germination (%G), the quality index and seedling vigour index (SVI-1 and SVI-11) in fresh and partially aged (1 year and 2 year) seeds of *Phyllanthus fraternus*. Comparative data suggest that the storage potential of seeds can be improved significantly with the help of PGRs.

KEYWORDS: Growth regulatory hormones, *Withania somnifera*, *Boerhaavia diffusa*, *Phyllanthus fraternus*.

INTRODUCTION:

The term plant hormone for growth regulating substances was introduced by Fitting (1909). The seed is one of the astonishing innovations of nature and it is now established that plant hormones affect seed germination by acting on different parts of the seed (Srivastava, 2002). Recent advances in agricultural research has suggested that the improvement in crop productivity and quality can be further improved by incorporating new technologies into traditional breeding programs and influence harvesting stages on seed vigour (Anonymous, 1985 & Mhatre and Rao, 1998). Grove *et al.* (1979) reported that brassinolide is a plant growth-promoting steroid, which they isolated from *Brassica napus* pollen. Tsai F-Y *et al.* (1997) made a comparative study of the effects of abscisic acid and methyl jasmonate, plant growth regulators, on seedling growth of rice. They found that growth regulators promote flowering, cellular division, and in seeds growth after germination. Gopikumar and Moktan (1994) studies the effects of plant hormones on seed germination and growth of true seedlings in the nursery. Tsai *et al.* (1997) studied the metabolism of gibberellins and suggested that gibberellins are important in seed germination affecting enzyme production that mobilizes food production used for growth of new cells. Saxena (1974) described the presowing hardening treatments improves crop production. Ethylene upregulate auxin biosynthesis in *Arabidopsis* seedlings to inhibition of root cell elongation (Swarup et al, 2007). Nitric oxide signaling in plants and help in the synthesis in the chloroplast (Shapiro, 2005 & Roszer, 2012). Keeping in view the aforesaid facts a study was taken to observe the seedling vigour of fresh and partially aged seeds (6 month to 2 year old seeds and to improve the seedling vigour with presoaking treatment of Gibberellic Acid (GA₃) and Kinetin (KIN) on germination.

METHODOLOGY:

Studies of germination are divided into different steps during 2005 (Agrawal and Dadlani, 1987);

(1) Requirements: These were as follows:

(1.1) Substratum: Germination filter paper was used for the method of 'Top of paper (TP)'.

(1.2) Plant Growth Regulators (PGRs) or Hormones: Seeds were treated with PGRs for better results.

- **Hormone soaking treatment:**

Dry seeds were soaked in different concentrations of gibberellic acid (GA₃), (10⁻⁵M to 10⁻⁷M), kinetin (KIN) (10⁻⁵M to 10⁻⁷M) and distilled water (DW). Amount of it was double then the seed weight (1 g seeds in 2 ml solution). Soaking period was for 5 hours. Seeds were removed from the solution and transferred to the tray, which was covered with filter paper and dried at room temperature till than original weight was obtained. Dry seeds were put for germination up to the final count. This period was 28-30 days for *Withania somnifera* seeds. The germination filter paper was changed frequently to prevent microbial growth.

- **Hormones preparation:**

(1) Stock solution of GA₃ (10⁻³ M): Accurately weighed 34.64 mg GA₃ was dissolved in 2 ml acetone and final volume was made up to 100 ml DW. Different concentrations (10⁻⁵M to 10⁻⁷M) were prepared from stock.

(2) Stock solution of KIN (10⁻³M): Accurately weighed 21.52 mg KIN was dissolved in 2 ml 1N NaOH and final volume was made up to 100 ml DW. Different concentrations (10⁻⁵M to 10⁻⁷M) were prepared from stock.

(1.3) Germination Room (Growth chamber): Seeds were germinated in sterilized chamber or room in which temperature and light was adjustable.

(2) Physiological factors: Different factors played important role in germination.

(2.1) Light: It was given continuous during germination period. Intensity of it was 500 lux from tube light.

(2.2) Temperature: Temperature was constant during germination and data were recorded at 25 ± 2°C.

(2.3) Humidity: Relative humidity of the room was 50-55%.

(3) Germination parameters: Different parameters were recorded as follows (ISTA, 1985 a,b):

(3.1) Normal seedling count: Seedling showed the continue development into satisfactory plants. Non-infected and intact seedlings were counted as normal.

(3.2) Abnormal seedling count: Damaged, deformed, unbalanced or decayed seedlings were counted as abnormal.

(3.3) Germination percentage (%G): It was calculated on the basis of the number of normal seedlings (Agrawal, 1987).

$$\%G = N / T \times 100$$

N = Number of normal seedlings, T = Total number of seeds kept for the germination

(3.4) Seedling length: It was recorded in cm and divided into 2 subparts; Root length & Shoot length

(3.5) Fresh and dry weight of seedlings: Average fresh weight (per seedling) was recorded. Normal seedlings were packed in blotting paper and kept in oven at $80 \pm 2^{\circ}\text{C}$ till constant dry weight was recorded.

(3.6) Quality Index (QI) or Speed of germination: The formula was given by Maguire (1962);

$$QI = \sum \text{Number of seeds germinated} / \text{Day of inspection}$$

(3.7) Seedling Vigour Index (SVI): It was calculated with that the help of two different formulas;

- SVI-I was given by Jayraj and Karivartharaju (1992) as follows:

$$SVI-I = \%G \times \text{Dry weight of seedling}$$

- SVI-II was given by Abdul Baki, James and Anderson (1973) as follows:

$$SVI-II = \%G \times \text{Total length of seedling}$$

RESULT AND DISCUSSION:

Plant hormones are considered effective molecule in development of seeds. The hormones affect seed germination and dormancy by acting on different parts of the seed. Walz *et al.* (2002) stated that there is a correlation of auxins and cytokinins in plant, known as a A/C= constant. They further held that a gene encoding a protein modify by the phytohormones, indoleacetic acid acting by modulating chromosomal transcription. Gibberelins include a large range of chemicals that are produced naturally within plants and are important in seed germination, affecting enzyme production that mobilizes food production used for growth of new cells (Agrawal and Dadlani,1987). Gopikumar and Moktan (1994) studies on the effects of plant hormones on seed germination and growth of true seedlings in the nursery and found that plant hormones are suitable to cover the dormancy due to storing of seeds and initiate

germination. Plant hormones like GA₃ and KIN act upon a responsive plant system by interaction the molecules and effect the morphological, physiological and biochemical responses. Chinoy (1942, 1967) had first time tried presowing treatment of PGRs in wheat seeds. The critical studies were made on proper concentration, soaking volume and application of PGRs on Indian plants (Saxena, 1974, 1990; Murlikrishna, 1993). In the present investigation the pretreated stored seeds were with 10⁻⁵ M to 10⁻⁷ M of GA₃ and KIN kept in distilled water for five hours gave good (Table 2 & Table 3). The results were better in comparison to control (Table 1). The present study is in conformity with the observations of earlier investigation in the field (Chinoy, 1942 & 1967; Saxena, 1974 & 1990; Murlikrishna, 1993). Out of three concentration of GA₃ and Kin (10⁻⁵M, 10⁻⁶M, 10⁻⁷M), GA₃ 10⁻⁵ M and KIN 10⁻⁷ M are best treatment for freshly and partially (1 year and 2 year) aged seeds of *Withania somnifera* (Var. WS 100), GA₃ 10⁻⁶ M and KIN 10⁻⁶ M are best treatment for freshly and partially (1 year and 2 year) aged seeds of *Boerhaavia diffusa* (Var. Local) and GA₃ 10⁻⁶ M and KIN 10⁻⁶ M are best treatment for freshly and partially (1 year and 2 year) aged seeds of *Phyllanthus fraternus* (Var. Local) to improve % G, SVI-I and SVI-II drastically. The PGRs are known to break seed dormancy in a number of plants (Gopikumar and Moktan, 1994).

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OBSERVATION TABLES:

Table 1.1: Seed vigour and germination data of WS 100 variety of *Withania somnifera*

Storage period	No. of seeds	Normal seedlings	Abnormal seedlings	% G	Average root length (cm)	Average shoot length (cm)	Average total length (cm)	Average fresh weight (mg)	Average dry weight (mg)	Q.I.	SVI - I	SVI - II
0 Month	25	14	0	56	1.5	1.0	2.5	21.73	0.65	1.35	36.40	148.0
6 Month	25	14	0	56	1.0	1.0	2.0	21.73	0.50	1.83	25.00	112.0
12 Month	25	14	0	56	0.7	1.2	1.9	21.42	0.39	1.54	21.84	106.4
18 Month	25	10	0	40	0.9	1.0	1.9	29.00	0.45	1.23	18.00	76.0
24 Month	25	9	0	36	0.9	1.0	1.9	29.44	0.49	0.93	17.64	68.4

Table 1.2: GA₃ treatment to Fresh and Partially aged seeds of WS 100 variety of *Withania somnifera*

Treatments	No. of seeds	Normal seedlings	Abnormal seedlings	% G	Average root length (cm)	Average shoot length (cm)	Average total length (cm)	Average fresh weight (mg)	Average dry weight (mg)	Q.I.	SVI - I	SVI - II
Treatment to 0 Month Old seeds (Fresh seeds)												
GA ₃ 10 ⁻⁵ M	25	15	0	60	3.3	2.2	5.5	34.60	0.67	2.67	40.20	330.0
GA ₃ 10 ⁻⁶ M	25	15	0	60	2.0	1.5	3.5	27.33	0.40	2.67	40.20	210.0
GA ₃ 10 ⁻⁷ M	25	12	3	48	1.2	1.2	2.4	25.90	0.36	2.12	17.28	115.2
Treatment to 12 Month Old seeds												
GA ₃ 10 ⁻⁵ M	25	12	0	48	1.4	1.2	2.6	25.00	0.62	1.85	29.76	124.8
GA ₃ 10 ⁻⁶ M	25	9	0	36	1.2	1.5	2.7	27.77	0.55	1.28	19.80	97.2
GA ₃ 10 ⁻⁷ M	25	6	0	24	1.0	1.5	2.5	42.50	0.48	0.86	11.52	60.0
Treatment to 24 Month Old seeds												
GA ₃ 10 ⁻⁵ M	25	5	1	20	3.0	1.0	4.0	52.00	0.70	0.20	14.00	80.0
GA ₃ 10 ⁻⁶ M	25	6	4	24	1.8	1.2	3.0	40.00	0.42	1.45	10.08	72.0
GA ₃ 10 ⁻⁷ M	25	6	0	24	1.0	1.2	2.2	28.33	0.52	0.69	12.48	52.8

Table 1.3: KIN treatment to Fresh and Partially aged seeds of WS 100 variety of *Withania somnifera*

Treatments	No. of seeds	Normal seedlings	Abnormal seedlings	% G	Average root length (cm)	Average shoot length (cm)	Average total length (cm)	Average fresh weight (mg)	Average dry weight (mg)	Q.I.	SVI - I	SVI - II
Treatment to 0 Month Old seeds (Fresh seeds)												
KIN 10 ⁻⁵ M	25	8	3	32	2.6	1.9	4.5	36.9	0.50	1.01	16.00	144.0
KIN 10 ⁻⁶ M	25	9	3	36	3.3	2.3	5.6	31.7	0.33	1.54	11.88	201.6
KIN 10 ⁻⁷ M	25	14	1	56	1.2	2.2	3.4	33.6	0.38	2.21	21.28	190.4
Treatment to 12 Month Old seeds												
KIN 10 ⁻⁵ M	25	6	4	24	1.8	1.3	3.1	40.0	0.43	1.40	10.32	74.4
KIN 10 ⁻⁶ M	25	5	1	20	3.0	1.1	4.1	52.0	0.71	0.23	14.20	82.0
KIN 10 ⁻⁷ M	25	12	3	48	1.2	1.0	2.2	25.9	0.36	2.12	17.28	105.6
Treatment to 24 Month Old seeds												
KIN 10 ⁻⁵ M	25	5	0	20	0.8	1.0	1.8	27.2	0.21	0.20	4.20	36.0
KIN 10 ⁻⁶ M	25	6	2	24	0.7	1.1	1.8	28.9	0.25	0.38	6.00	43.2
KIN 10 ⁻⁷ M	25	7	0	28	0.9	1.6	2.5	35.0	0.40	0.52	11.20	70.0

Table 2.1: Seed vigour and germination data of Local variety of *Boerhaavia diffusa*

Storage period	No. of seeds	Normal seedlings	Abnormal seedlings	% G	Average root length (cm)	Average shoot length (cm)	Average total length (cm)	Average fresh weight (mg)	Average dry weight (mg)	Q.I.	SVI - I	SVI - II
0 Month	25	17	0	68	4.4	1.5	5.9	13.1	1.03	6.2	70.1	398.1
6 Month	25	16	0	64	3.2	1.0	4.2	10.1	0.94	3.1	61.2	276.9
12 Month	25	18	0	72	1.5	2.0	3.5	9.2	0.83	3.3	59.2	251.0
18 Month	25	16	0	64	1.5	1.8	3.3	8.9	0.78	3.2	50.4	210.1
24 Month	25	15	3	60	1.8	2.0	3.8	3.9	0.33	4.3	20.1	226.0

Table 2.2: GA₃ treatment to Fresh and Partially aged seeds of Local variety of *Boerhaavia diffusa*

Treatments	No. of seeds	Normal seedlings	Abnormal seedlings	% G	Average root length (cm)	Average shoot length (cm)	Average total length (cm)	Average fresh weight (mg)	Average dry weight (mg)	Q.I.	SVI - I	SVI - II
Treatment to 0 Month Old seeds (Fresh seeds)												
GA ₃ 10 ⁻⁵ M	25	23	0	92	1.4	1.9	3.3	4.5	0.43	2.8	40.0	299.1
GA ₃ 10 ⁻⁶ M	25	24	0	96	2.0	1.7	3.7	7.9	0.83	3.9	81.3	351.2
GA ₃ 10 ⁻⁷ M	25	22	0	88	1.1	1.2	2.3	3.8	0.34	3.0	30.2	209.0
Treatment to 12 Month Old seeds												
GA ₃ 10 ⁻⁵ M	25	18	1	72	1.2	1.6	2.8	9.3	0.83	3.1	60.1	203.1
GA ₃ 10 ⁻⁶ M	25	18	3	72	3.5	1.5	5.0	6.5	0.56	3.3	40.1	359.9
GA ₃ 10 ⁻⁷ M	25	16	0	64	1.5	1.8	3.3	8.9	0.78	3.2	50.4	210.1
Treatment to 24 Month Old seeds												
GA ₃ 10 ⁻⁵ M	25	18	2	72	1.7	1.8	3.5	7.2	0.69	3.35	51.3	255.1
GA ₃ 10 ⁻⁶ M	25	16	3	64	3.2	1.1	4.2	10.1	0.94	3.1	61.2	276.9
GA ₃ 10 ⁻⁷ M	25	15	3	60	1.8	2.0	3.8	3.9	0.33	4.3	20.1	226.0

Table 2.3: KIN treatment to Fresh and Partially aged seeds of Local variety of *Boerhaavia diffusa*

Treatments	No. of seeds	Normal seedlings	Abnormal seedlings	% G	Average root length (cm)	Average shoot length (cm)	Average total length (cm)	Average fresh weight (mg)	Average dry weight (mg)	Q.I.	SVI - I	SVI - II
Treatment to 0 Month Old seeds (Fresh seeds)												
KIN 10 ⁻⁵ M	25	23	0	92	1.0	1.2	2.2	6.3	0.54	3.3	50.9	201.3
KIN 10 ⁻⁶ M	25	23	0	92	2.2	1.9	4.1	5.2	0.43	5.2	40.1	377.1
KIN 10 ⁻⁷ M	25	23	0	92	1.4	1.9	3.3	4.5	0.43	2.8	40.0	299.1
Treatment to 12 Month Old seeds												
KIN 10 ⁻⁵ M	25	22	0	88	1.1	1.2	2.3	3.8	0.34	3.0	30.2	209.0
KIN 10 ⁻⁶ M	25	22	1	88	1.4	1.7	3.1	6.8	0.57	3.0	51.2	274.9
KIN 10 ⁻⁷ M	25	22	1	88	1.1	1.2	2.3	7.1	0.52	3.0	48.2	203.1
Treatment to 24 Month Old seeds												

KIN 10^{-5} M	25	16	0	64	1.5	1.8	3.3	8.9	0.78	3.2	50.4	210.1
KIN 10^{-6} M	25	17	0	68	4.4	1.5	5.9	13.1	1.03	6.2	70.1	398.1
KIN 10^{-7} M	25	18	4	72	1.0	1.8	2.8	5.1	0.42	3.3	31.3	198.8

Table3.1: Seed vigour and germination data of Local variety of *Phyllanthus fraternus*

Storage period	No. of seeds	Normal seedlings	Abnormal seedlings	% G	Average root length (cm)	Average shoot length (cm)	Average total length (cm)	Average fresh weight (mg)	Average dry weight (mg)	Q.I.	SVI - I	SVI - II
0 Month	25	23	0	92	1.0	1.0	2.0	1.8	0.14	6.00	12.8	181.1
6 Month	25	19	0	76	1.2	1.2	2.4	3.01	0.21	5.50	15.9	181.2
12 Month	25	13	0	52	1.1	2.3	3.4	2.49	0.25	3.75	12.8	176.1
18 Month	25	15	0	60	1.1	1.4	2.5	2.5	0.22	4.50	13.1	151.0
24 Month	25	15	0	60	1.2	1.1	2.3	2.4	0.22	4.75	13.0	141.0

Table 3.2: GA₃ treatment to Fresh and Partially aged seeds of Local variety of *Phyllanthus fraternus*

Treatments	No. of seeds	Normal seedlings	Abnormal seedlings	% G	Average root length (cm)	Average shoot length (cm)	Average total length (cm)	Average fresh weight (mg)	Average dry weight (mg)	Q.I.	SVI – I	SVI - II
Treatment to 0 Month Old seeds (Fresh seeds)												
GA ₃ 10^{-5} M	25	17	0	68	1.0	1.4	2.4	2.2	0.20	4.1	13.5	159.1
GA ₃ 10^{-6} M	25	23	0	92	1.0	1.0	2.0	1.8	0.14	6.0	12.8	181.1
GA ₃ 10^{-7} M	25	17	0	68	1.1	1.1	2.2	2.1	0.19	4.0	13.1	151.8
Treatment to 12 Month Old seeds												
GA ₃ 10^{-5} M	25	16	0	64	1.2	0.8	2.2	1.7	0.13	2.2	8.1	124.8
GA ₃ 10^{-6} M	25	18	0	72	1.2	0.7	1.9	2.0	0.17	3.5	12.5	141.0
GA ₃ 10^{-7} M	25	17	0	68	1.2	0.6	1.8	1.8	0.12	2.0	8.1	125.1
Treatment to 24 Month Old seeds												
GA ₃ 10^{-5} M	25	9	0	36	1.2	0.9	2.1	2.6	0.22	3.0	8.2	75.1
GA ₃ 10^{-6} M	25	15	0	60	1.1	1.4	2.5	2.5	0.22	4.5	13.1	151.0
GA ₃ 10^{-7} M	25	11	0	44	1.0	1.3	2.3	1.5	0.11	2.2	5.1	99.8

Table 3.3: KIN treatment to Fresh and Partially aged seeds of Local variety of *Phyllanthus fraternus*

Treatments	No. of seeds	Normal seedlings	Abnormal seedlings	% G	Average root length (cm)	Average shoot length (cm)	Average total length (cm)	Average fresh weight (mg)	Average dry weight (mg)	Q.I.	SVI - I	SVI - II
Treatment to 0 Month Old seeds (Fresh seeds)												
KIN 10 ⁻⁵ M	25	15	0	60	1.2	1.1	2.3	2.4	0.22	4.75	13.0	141.0
KIN 10 ⁻⁶ M	25	17	0	68	1.1	1.4	2.4	2.2	0.20	4.1	13.5	159.1
KIN 10 ⁻⁷ M	25	18	0	72	1.2	0.7	1.9	2.0	0.17	3.5	12.5	141.0
Treatment to 12 Month Old seeds												
KIN 10 ⁻⁵ M	25	10	0	40	1.0	1.1	2.1	2.1	0.18	2.00	7.1	85.1
KIN 10 ⁻⁶ M	25	15	0	60	1.2	1.1	2.3	2.4	0.22	4.75	13.0	141.0
KIN 10 ⁻⁷ M	25	11	0	44	1.1	1.0	2.1	1.9	0.14	2.00	6.2	91.0
Treatment to 24 Month Old seeds												
KIN 10 ⁻⁵ M	25	7	0	28	1.0	1.1	2.1	1.9	0.14	3.5	4.1	61.0
KIN 10 ⁻⁶ M	25	10	0	40	1.1	1.1	2.1	2.1	0.18	2.00	7.1	85.1
KIN 10 ⁻⁷ M	25	6	0	24	1.1	1.8	2.9	2.0	0.17	1.75	4.2	71.2