

IMPACT OF CHEMICAL PRE-TREATMENTS ON SEED GERMINATION AND SEEDLING GROWTH OF ABRUS PRECATORIUS L.

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ABSTRACT

Abrus precatorius L. is commonly known as Gunja belonging to family Fabaceae , abundantly found all throughout the plains of India, from Himalaya down to Southern India. This plant is having medicinal potential to cure various diseases. The roots, leaves and seeds of this plant are used for different medicinal purpose. Seeds of *Abrus precatorius* posses seed dormancy and restricts germination to overcome unfavorable environmental conditions. This dormancy need to be removed to improve seed germination under favourable condition of plant improvement. So the aim of the study is to remove seed dormancy and improve germination capacity within a short period. To overcome the problem of dormancy, the experiment was carried out to investigate the effect of different treatments like 2,4-Dichlorophenoxyacetic acid, kinetin, Sulfuric acid ,Hydrochloric acid on germination and seedling growth. Experimental design was randomized complete block design with 3 replications. It was observed that the seeds of *Abrus precatorius* (white) treated with 2,4-Dichlorophenoxyacetic acid 75 ppm (70 %), kinetin, 75 ppm (73%) were proved favorable to express maximum percent germination. Seeds soaked in Sulfuric acid for 2min (70%) found maximum percent germination.

Key words: *Abrus precatorius*, Germination, root length and shoot length.

INTRODUCTION.

Abrus Precatorius is one of the important herb belonging to family Fabaceae, Ratti (Hindi). India has the richest plant diversity in the world, many of which are medicinally valuable. The rich resource is decreasing at an alarming rate as a result of over-exploitation. Among the traditional system of medicine [1].This plant can be easily identified with different colored hard seed coated seeds. The unique characteristic of this plant is that it has toxic red seeds with black mark at the base [2].Seeds are said to be antiphlogistic, emetic, tonic, purgative, anti-ophthalmic and aphrodisiac. Seed of this plant are very beautiful and they attract children. These seed are used to make Necklaces and other ornaments. Leaves and seeds are nutritious as boiled seeds are eaten in certain parts of India. It is said that cooking destroys the poison of seeds [3], [4]. Dry seeds of *A. precatorius* are used to cure worm infection. In veterinary medicine, it is used

in the treatment of fractures. Seeds have also the potential of good insecticide and antimicrobial activity. Various African tribes use powdered seeds as oral contraceptives. Abrus seeds are also taken for tuberculosis and painful swellings^[5]. Seeds are rich in several essential amino acids like serine, Abrusin, Abrusin-2'-O-apioside, hederagenin, sophoradiol, sophoradiol-22-O-acetate, tryptophan^[6] trimethyl^[7] alanine^[8] amyrin, alpha, ursolic acid^[9] valine^{[10], [11]} and methyl ester. India is one of the largest producers of herbs and herbal products. Nature around us provided everything of necessity of mankind^[12]. The large resources of medicinal plants have been used continuously for the treatment of different diseases^[13]. Medicinal plants can be important source of previously unknown chemical substances with potential therapeutic effects. The world health organization (WHO) has estimated that over 75% of the world's population still relies on plant derived medicines, usually obtained from traditional healers, for its basic health care needs^[14]. Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs^[15].

For the threatened or endangered state of many of the plants, many factors, such as human settlement, natural calamities, unscientific exploitation and road construction were responsible^[16]. On the today's market, demand is increasing and the supply of herbal drugs is decreasing. Thus, to fulfill the gap between demand and supply, there is an urgent need to conserve and cultivate medicinally important plant species^{[17], [18]}. The seed, being the potential means of propagation, needs to be extensive study for its germination requirements, at first.

Plant growth regulators are the organic compounds other than nutrients which effect the morphological structure and physiological processes of plants in low concentrations. Plants hormones are naturally occurring growth hormones which in low concentrations control physiological processes in plant. More commonly the term plant growth regulators are used because it includes both the native and synthetic substances which modify the plant growth. The five major kinds of substances are auxins, gibberellins, cytokinins, abscisic acid and ethylene. In general the plant growth regulators serve in regulating cell enlargement, cell division, cell differentiation, organogenesis, senescence and dormancy. They are employed in seed treatment to achieve earlier growth and root development^[19].

The present study observed the effectiveness of different seed dormancy breaking treatments to improve early germination of white seeded variety of *Abrus precatorius*. In the wild conditions, seeds takes at least one year for the germination. The seed, being the potential source of propagation, needs to be extensive study for its germination. This facts indicate the existence of seed dormancy in this plant and no literature is available describing the seed dormancy breaking methods to bring about early germination in white *Abrus precatorius*. With these objectives, to observe the effect of 2,4-Dichlorophenoxyacetic acid, Kinetin, Sulfuric acid and Hydrochloric acid on seed germination and seedling growth of *Abrus precatorius* (white) the present study was planned.

MATERIALS AND METHODS.

This study was conducted at the Experimental Laboratories of Department of Botany. Swami Vivekanand senior college Mantha, Dist.Jalna (M.S) India.

A) Collection Of Seed Material.

In the present study, seeds of the *Abrus precatorius* (White) were collected from different locations of Jalna district. Collected seeds were then packed in sterile polythene bags in first week of June 2019.

B) Treatments of Seeds.

Seeds were first surface sterilized for 1 minute in 0.1 % HgCl₂ solution for 5 minutes and subsequently washed with water. The experiment was arranged as a completely randomized design with three replications for each treatment. Seeds were treated with T1. (2,4-D) = 2, 4-Dichlorophenoxyacetic acid; T2. (Kn) = kinetin. For 25ppm, 50ppm,75ppm,100ppm and 200ppm.T3 - (H₂SO₄) = Sulfuric acid (80%) , T4= (HCL)= Hydrochloric acid (80%) for 2 min,4min,6min,8min and 10min.Germination was measured daily for 60 days. All plants were harvested to determine percent germination, shoot length, root length and number of leaves.

C) Identification of fungi.

The fungi occurring on nongerminated seed parts in the plates were identified preliminary on the basis of sporulation characters like sexual or asexual spores with the help of stereoscopic binocular microscope. The identification and further confirmation of fungi was made by preparing slides of the fungal growth and observing them under compound microscope. The identification was made with the help of manuals [20] Similarly confirmation of identification was made at Department of Plant Pathology Laboratory, Dr. Babasaheb Ambedkar Marathwada University Aurangabad. Pure cultures of these fungi were prepared and maintained on potato dextrose agar (PDA) slants.

D) Statistical Analysis.

In the present study data was analyze statistically, Standard Deviation (S.D) Standard Error (S.E) and Critical Difference (C.D) was calculated.

EXPERIMENTAL RESULTS

The effect of different treatments like T1. (2,4-D) = 2, 4-Dichlorophenoxyacetic acid; T2. (Kn) = kinetin. for 25ppm, 50ppm,75ppm,100ppm and 200ppm.T3 - (H₂SO₄) = Sulfuric acid (80%) , T4= (HCL)= Hydrochloric acid (80%) for 2 min,4min,6min,8min and 10min presoaking treatment on seeds of *Abrus precatorius* (white) were treated. Percent germination, shoot length, root length and number of leaves were observed.

The results are mentioned in Table 1, 2 and 3. It is clear from result summarized in table 1 that the seeds of *Abrus precatorius* (white) treated with 2, 4-Dichlorophenoxyacetic acid 75 ppm (70 %) were proved favorable to express maximum percent germination, shoot length and root length. In case of kinetin, 75 ppm were proved favorable to express maximum percent germination (73%). Kinetin for 50ppm proved favorable to express maximum root length (17.81) as compared with control. All results are statistically significant.

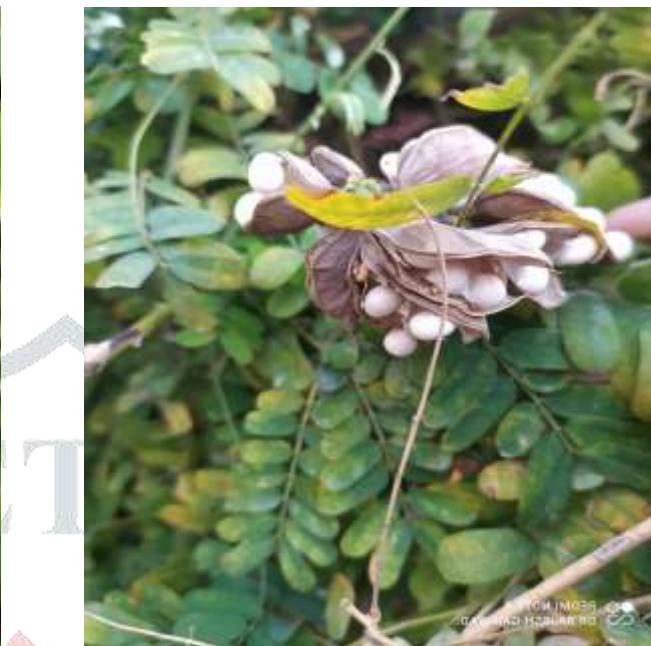
It is clear from Table 2 that treatment with H₂SO₄ and HCL was effective in breaking the seed dormancy and the result is shown in Table 2. Seeds soaked in Sulfuric acid (80%) for 2min gave the highest germination of 70% and shoot length (12.71). The treatment with Hydrochloric acid (80%) for 4 min gave 70% germination and for 6 min gave maximum shoot length (15.24), but none of the seeds germinated after soaking for 8 min and 10 min as compared with control for the period of the experiment. In order to study the incidence of infection of fungi on infected seeds of *Abrus precatorius* the experiment was conducted.

The results are mentioned in table 3. In 2, 4-Dichlorophenoxyacetic acid chemical treatments, 05 different types of fungi was found to be observed namely *Alternaria alternata*, *Aspergillus niger*, *A. fumigatus*, *Aspergillus flavus*, *Mucur* spp, and seeds treated by kinetin treatments 05 different types of fungi was found namely *Aspergillus niger*, *A. fumigatus*, *Aspergillus flavus*, *Rhizopus stolonifer*, *Mucur* spp similarly In Sulfuric acid (2 min) (80%) chemical treatments, 02 types of fungi was found to be observed namely *Aspergillus niger* and *Mucur* spp, Seeds treated by Hydrochloric acid (80%)treatments 02 different types of fungi was found namely *Aspergillus niger* and *Mucur* spp.

Table 1. Effect of plant growth hormones (24-D and Kn) on seed germination and seedling growth of *Abrus precatorius*.

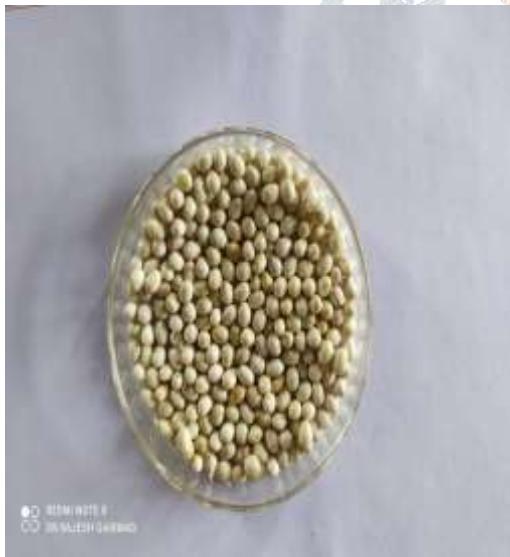
Treatment (ppm)	<i>Abrus precatorius</i> .							
	Germination (%)		Mean					
			Shoot length (cm)		Root length (cm)		No. of leaves	
	T1	T2	T1	T2	T1	T2	T1	T2
25	50	50	13.41	12.29	8.89	9.77	8.64	7.14
50	50	63	13.64	13.54	9.20	17.81	7.61	7.61
75	70	73	13.88	15.71	12.32	10.79	8.81	8.37
100	53	70	10.39	12.87	8.86	10.52	9.12	7.55
200	--	50	--	11.66	--	9.45	--	7.91
Control (Pre-Soaked)	46	40	7.82	6.33	5.66	5.25	5.44	7.41
S.D	9.39	13.00	2.72	3.14	2.36	1.78	1.50	0.43
S.E	4.20	5.31	1.11	1.28	1.05	1.26	0.67	0.17
C.D	10.79	13.64	2.89	3.29	2.71	1.86	1.72	0.45

T1. (2,4-D) = 2, 4-Dichlorophenoxyacetic acid, T2. (Kn) = kinetin.

PLATE 1. ABRUS PRECATORIUS L. PLANT PARTS

1. Flowers

2. Seeds



3. Healthy Seeds

4. Effect of 2,4-D on seeds of *Abrus precatorius*.

Table 2. Effect of (H₂SO₄ and HCL) treatments on seed germination and seedling growth of *Abrus precatorius*.

Treatment (Min.)	<i>Abrus precatorius.</i>								
	Germination (%)		Shoot length (cm)		Root length (cm)		Mean No. of leaves		
	T3	T4	T3	T4	T3	T4	T3	T4	
2	70	56	12.71	11.91	9.11	8.34	10.44	8.64	
4	66	70	11.40	12.84	9.18	8.23	8.32	7.61	
6	50	69	10.87	15.24	9.32	9.87	10.77	8.81	
8	--	50	--	10.41	--	8.32	--	7.12	
10	--	--	--	--	--	--	--	--	
Control (Pre-Soaked)	30	30	9.91	11.71	8.23	8.20	8.28	5.44	
S.D	10.42	9.80	1.17	1.80	0.49	1.82	1.34	1.36	
S.E	5.22	4.42	0.58	0.80	0.25	0.79	0.67	0.61	
C.D	13.34	11.28	1.50	2.07	0.64	2.11	1.72	1.56	

T3 - (H₂SO₄) = Sulfuric acid (80%), T4- (HCl)= Hydrochloric acid (80%).**Table 3: Incidence of fungi on infected seeds of *Abrus precatorius*.**

Fungi	<i>Abrus precatorius.</i>				
	Incidence of fungi on seeds treated by				
	T1	T2	T3	T4	T5
<i>Alternaria alternata</i>	+	--	--	--	+
<i>Aspergillus niger</i>	+	+	+	+	+
<i>Aspergillus fumigatus</i>	+	+	--	--	+
<i>Aspergillus flavus</i>	+	+	--	--	+
<i>Rhizopus stolonifer</i>	--	+	--	--	+
<i>Mucur</i> spp	+	+	+	+	+

--=Absent + = Present.

T1- (2,4-D) = 2, 4-Dichlorophenoxyacetic acid, T2- (Kn) = kinetin, T3-(H₂SO₄) (2 min) = Sulfuric acid (80%), T4- (HCl)= Hydrochloric acid (80%), T5- Control (pre-soaked).

DISCUSSION.

Treatment with H₂SO₄ and HCl was effective in breaking the seed dormancy. Seeds soaked in Sulfuric acid and Hydrochloric acid concentrated acid for 3 min gave the highest germination of 50%. The treatment with 90% concentration of the acid for 3 min gave 28.6% germination, but none of the seeds germinated after soaking for 5 min. No germination was recorded from seeds in the control for the period of the experiment. Total germination of seeds mechanically scratched in gravel was 21.4% and no germination from the control. Treatment of seeds for 4 sec in hot water gave the highest germination of 42.9%.^[21] Seed sample recorded the purity of seed (97.55 %) and seed sample showed the maximum germination percentage 95% after three and four hrs, scarification. The maximum root length (34.07 mm),

maximum shoot length (23.62 mm) and maximum seedling shoot and root fresh weight (0.23 and 0.24 g) were observed at three, two hrs and control. H₂SO₄ scarification. The results indicated that H₂SO₄ scarification increase the germination percentage but it reduce the viability of the seed^[22]. Improved the seed germination when reported that pre-treated the *Glycyrrhiza glabra* seeds with concentrated H₂SO₄ for five min improved the germination.^[23] Seeds of *Cassia angustifolia* treated with H₂SO₄ for 12 min gave highest germination of 72 per cent.^[24] On the other hand could not find any improvement in germination of *Catharanthus roseus* seeds due to pre-soaking treatments for different periods.^[25]

Treatment with 50% HCL concentration showed effectiveness in breaking dormancy of *Parkia biglobosa*. The seeds soaked in 50% HCL concentration for 30 minute gave the germination percentage of 70% in 21 days period of experiment. HCL of 50% concentration gave a germination percentage of 70% in just 20 days. HCL was effective in breaking the dormancy of seeds of Parkia biglobosa^[26]. External supply of growth hormones (GA₃, Kinetin, KNO₃) to seeds, help in the activation of enzyme responsible for the breaking down of reserved food materials and also counteract the inhibitors present in the seed there by facilitating the germination^{[27], [28]}. Immersion of seed in concentrated acid disrupts the seed coat, the fact that 50% HCL concentration gave 70% of germination percentage within 13 day after sowing. This indicates that acid therefore disrupt the seed coat and expose the lumen of the macrosleids cells, permitting imbibitions of water which triggers germination^[29]. Pre-treatments of the seeds with different acids (H₂SO₄) indicated that rupture of seed coat can easily accelerate the germination process. In chemical treatments, the maximum germination percent was observed after pre-treatment the seeds with H₂SO₄ for 10 min by 57.33% followed by H₂SO₄ for 5 min (39.66%) On the other hand, lowest germination was obtained in HCl for 5 min by 7.33^[31].

CONCLUSIONS.

Pre-sowing treatment of seed plays important role to enhance the seed germination under nursery conditions. Among the pre-sowing treatments, the best treatment for the sowing of *Abrus precatorius* (White) seeds are treated with 2,4-Dichlorophenoxyacetic acid 75 ppm (70 %), kinetin, 75 ppm (73%) were proved favorable to express maximum percent germination. Seeds soaked in Sulfuric acid for 2 min found maximum percent germination (70%) may be recommended for plantation programme.

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