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Seed germination improvement in three important medicinal plant species *Abelmoschus moschatus* (Medik), *Asparagus racemosus* (Willd), *and Cassia angustifolia* (Linn)

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Abstract
In the present study, dormancy and germination requirement were investigated in one year old and fresh seeds of three important medicinal plants are *Asparagus racemosus, Cassia angustifolia, Abelmoschus moschatus* seeds were subjected to 12 pretreatments in the present study. There was Significant result on fresh seeds of *Abelmoschus moschatus* both under *in vitro* and *in vivo* conditions. Seed germination recorded from one year old seed treated with most of the treatments was at par with untreated control. However, water soaking for 24 hrs, Gibbrellic acid and sand paper scarification had higher per cent seed germination on fresh seed under *in vitro* and on both fresh as well as one year old seeds under *in vivo* conditions. *In vivo*, maximum percent of 86.50 was recorded from fresh seed soaked only in water for 24 hrs whereas, in control, percent seed germination was 30.0.Most of the treatments did not have any effect on seed germination on fresh and one year old seed of *Asparagus racemosus* under *in vitro* and *in vivo* conditions. However, Hot water treatment (70 ^{0}C) for 1hr had significant positive effect on one year old seed germination (47.00) closely followed by Cow dung Water + 24 hrs water Soaking (76.00). Moreover, cow dung water + 24 hrs Gibbrellic acid solution and cow dung + water Soaking for 24 hrs did also have significant positive effect. *Cassia angustifolia*, sand paper scarification + 24 hrs water soaking showed promising results significantly enhancing seed germination of on fresh seed (64.00) under *in vitro* condition and on one year old seed (57.00) under *in vivo* conditions. Whereas in control, seed germination of 34.67 and 23.50 per cent respectively recorded from fresh and one year old seeds.

Keywords: *Abelmoschus moschatus, Asparagus racemosus, Cassia angustifolia,* dormancy, germination, treatments

Introduction
Roots of *Asparagus racemosus* (Shatavari; Family: *Asparagaceae*) is used as a remedy for tuberculosis, leprosy, epilepsy, dysentery, tumors, inflammations and night blindness. Fresh juice from tuber is given orally in dysentery, acidity and to increase the breast milk after delivery. Medicated oil prepared from tubers is beneficial for nervous and rheumatic complaints (Khare 2007) [9]. Therapeutic use of Asparagus racemosus include cooling and bitter herb is also known for its anti-inflammatory qualities and may be used in infections such as cystitis and dysentery. Shatavari's mild diuretic action addresses the need in bladder infections, an antacid and demulcent. Satavari is effective in ulcers and hyperacidity and its cooling action works on chronic fevers, inflamed membranes of the lungs, Stomach, Kidneys and Sexual organs (R. Freeman, 1998) [13]. It also used as a nervinetonic, Antilithiaticeffects, Antioxidant effects (Takeungwongtrakul, 2012) [15]. Antineoplastic activity, Antitussive effect, Antidepressant activity, It helps with nervousness, pain, restless sleep, disturbing dreams and people with weak emotional and physical heart [9]. The dried roots of the plant are used as drug. The roots are said to be tonic and diuretic and galactgogue, the drug has ulcer healing effect, probably via strengthening the mucosal resistance or cytoprotection. It has also been identified as one of the drugs to control the symptoms of AIDS. *Cassia angustifolia* (family *Caesalpiniacea*) popularly known as Senna, is a valuable plant drug in Ayurvedic and modern system of medicine for the treatment of constipation (Atal and Kapoor, 1982; Das et al., 2003; Martindale, 1977; Sharma, 2004) [4, 5, 10, 14]. Senna is a sun-loving crop and requires bright sunshine for its successful growth. The crop is raised from seed and has a hard and tough seed-coat, ascertain amount of abrading of its surface is necessary to induce quick germination.

The Sennosides had been extracted from Senna leaves, stems, pods, buds and flowers but no Sennosides were found in the seeds. Cultivation of Senna does not require much expenditure on irrigation, manuring, pesticides, protection and other pre- and post-harvest care. This makes the plant ideal crop for acid regions where water provision, wasteland development; desertification control and sand dune stabilization are the major challenges (Tripathi, 1999) [17, 18].

Material and Methods

Seed source and collection of seeds: Fresh seeds of *Abelmoschus moschatus*, *Asparagus racemosus, Cassia angustifolia,* were collected from medicinal plant nursery which is situated in inside the college campus of of Thakur Chhedilal Barrister College of Agriculture and Research Station, Bilaspur, Chhattisgarh, India lies in 22°9'12" North latitude and 82° 12'12" East longitude at South Eastern Central Zone of India. *And seeds were packed in air tight* plastic box at room temperature for one year. In this process after one year later seeds were collected again from this nursery which is used in the fresh seed form.

Experiments: experiment were lies in the laboratory of the Agriculture Plant pathology laboratory of Thakur Chhedilal Barrister College of Agriculture and Research Station, Bilaspur, Chhattisgarh. The experiment content two factors i.e. seed age (fresh seeds and one-year-old seeds) and treatments under both sowing conditions i.e. *in vitro* (Laboratory conditions) and *in vivo* (Nursery bed). Following treatments were applied in the present study.

Treatment Code	Details of Treatment
T-1	Water Soaking for 24 h
T-2	Gibbrellic acid solution @ 10 mg/lit for 24 h
T-3	Sand Paper Scarification +Water Soaking for 24 h
T-4	Sulphuric acid (H_2SO_4 95%) for 1 h + Water Soaking for 24 h
T-5	Potassium nitrate (0.2%) for 24 h
T-6	Hydrochloric acid (HCl 35%) for 1 h + Water Soaking for 24 h
T-7	Nitric acid (HNO_3 63.1%) for 1 h + Water Soaking for 24 h
T-8	Hot Water (70 °C) + Water Soaking for 24 h
T-9	Cow dung Water + Water Soaking for 12/12 h
T-10	Cow dung Water + Gibbrellic acid solution @ 10 mg/lit for 12/12 h
T-11	Hot Water (70 °C) 1h + Water Soaking for 12/12 h
T-12	Cow dung + Water Soaking for 12/12 h
T-13	Control (Untreated)

Mechanical scarification with sandpaper

Seed coats were scarified manually with sandpaper for 1 minute and then the seeds were soaked in normal tap water for 24 h at room temperature.

Chemical Treatments

Seeds were soaked separately in H2SO4 (95%), Nitric acid (HNO3 63.1%) and Hydrochloric acid (HCl 35%) for 1 h then washed thricely with tap water and soaked in normal distilled water for 24 h. Gibbrellic acid (10 mg/l) and Potassium nitrate (0.2%) were used for 24 h presoaking of seeds at room temperature.

Hot Water Treatment

The seeds were placed in a cotton cloth bag and were kept in water bath at 70 °C for 1 h followed by keeping in water for 24 h at room temperature.

Hot Water followed by chemical Treatments

Seeds were placed in a cotton cloth bag and were kept in water both at 70 °C for 1 h. Further seeds were soaked in Gibbrellic acid solution (10 mg/l) for 24 h.

Cow dung water Treatments

Fresh cow dung was dried under sunlight for three days followed by autoclaved at 121.6 °C. 100 g of sterilized cow dung was added to one liter autoclaved water. It was swirled and mixed thoroughly. After settlement water was decanted and seeds were soaked in this solution for 12 h. After 12 hours, seeds were placed in Gibbrellic acid solutions for another 12 h as per the requirement of treatments.

Cow dung Treatment

The seeds were kept in fresh cow dung for 12 h there after seeds were transferred to sterilized water for another 12 h.

Experimental design, data parameter and analysis

Observations were recorded on germination in both conditions *in vitro* and *in vivo*. Different treatment combinations were applied to break seed dormancy using mechanical, physical and chemical methods. Entire experiment was replicated thrice and kept *in vitro* and germination (%) was recorded after three days of incubation and continued for 18 days. Where *In vivo* were sown in nursery beds there was data recorded after 15 days of sowing and continued up to 45 days. In these experiments we are using two factors Completely Randomized Design. The data recorded was subjected to statistical analysis using MS-tat software program.

Result and Discussion

The mechanically scarified seeds had a significant impact on breaking the dormancy. mechanically scarified for 2minute through sand paper and soaked in normal tap water for 24 hours The fresh Seeds of *Abelmoschus moschatus* and *Cassia angustifolia* showed 73.33% and 64.00%(*in vitro*) germination percentage respectively, whereas one year old seeds of *Cassia angustifolia* showed 57.00% germination percentage *in vivo* condition only. (Thilakar S.J. & R. Jeya, 2013) [16] also reported 100% seed germination in *Adenanthera pavonina* in mechanically scarification treatment.

In our present study mechanically scarified seeds showed very high germination percentage when compared to any other treatment. Mechanical scarification was very effective in overcoming dormancy in seeds of *Abelmoschus moschatus* and *Cassia angustifolia* the study of (Okunlola et al., 2011) [1] revealed that seeds mechanically scarified improved seed germination and seedling growth. It is therefore recorded that seeds mechanically scarified with sandpaper had germination of 83.3% in *P. biglobosa* (Okunlola et al., 2011) [1]. This shows that mechanical scarification may be effective for

breaking dormancy and improving the seedling vigor.

The applications of growth regulators GA310mg/l have been extensively used for enhancing the growth and development of seedlings under laboratory and nursery conditions. Fresh seeds of *Abelmoschus moschatus* presented 63.33% *in vitro* and 67.50%*in vivo* germination percentage In comparison to their control. In same treatment Fresh seeds of *Cassia angustifolia* showed 48.67% germination *in vitro* only, where no observed significant effect on Asparagus racemosus of these treatments.

Gibberellins are most prominent growth regulator, which are widely used in cultivated as well as in wild plants. (Nadjafi et. al. 2006) [11] Observed highest germination percentage in Teucrium polium when pretreated with 500-2500 ppm GA3. (Kasera et. al. 2011) [8] Observed maximum germination in *Leptadenia reticulata* with 10 and 25 mgl-1 of GA3. Data obtained from present studies clearly reveals that 5 and 10 mgl-1 GA3 pretreatments are suitable for obtaining maximum germination and seedling growth.

Hot water at 70 °C for 1 h followed by keeping seeds in water for another 24 h at room temperature had a positive effect on germination of A. racemosus germination increased from 10% to 46.5% under *in vivo* probably due to softened hard and thick seed coat. But there were the long duration contact of seed with hot water results damage of embryo. Similar result was also reported by Amusa (2011) [2] in A. africana when seeds were treated with 100 °C hot water for 12 h.

Acid scarification is also found out in the best dormancy breaking method which is analyzed from many scientists in their research study. Water impermeability of the testa is a physical exogenous dormancy according to (Nikolaeva 1969) [12]. Concentrated sulphuric acid has been used for many years for softening of hard seed coats (Hopkins, 1923). Germination test are based on pure seed components, this has been shown by the observations recorded and that purity analysis and germination tests complement each other. But there were in our study all three acids did not given any significant result as compare to another treatments as well as did not affected seed age also there was germination recorded in a range of 12.00% to 26.5%.

Fresh seeds of *A. moschatus*, *C. angustifolia* (*in vitro*), showed significantly higher germination than old seed. These variations in germination may be related to the types of seed coat and level of dormancy in different medicinal plants. Freshly collected seeds might have great potential while they are slightly immature will have thinner seed coats often germinates better (Asi *et al*., 2011) [3] resembles to our findings. One year aged seeds might accumulate some chemical substance in seed coat after exposure to environment, which probably makes them impervious, harder, results poor germination. However A. racemosus shown higher germination employing aged seeds, probably due to undeveloped embryo, which required some time to be mature and have extra advantages, Similarly variations on germination existed between *in vitro* and *in vivo* condition of the experiment among species and age of the seedlings.

Table 1.1: Effect of different treatments, on seed germination of important medicinal plant species *Cassia angustifolia.*

Treatments	*In vitro*			*In vivo*		
	Fresh	**1yr old**	**Mean**	**Fresh**	**1yr old**	**Mean**
Water soaking for 24 hrs	39.33	11.33	25.33	13.50	32.50	23.00
Gibbrellic acid @10,000 ppm	48.67	12.00	30.33	13.00	35.00	24.00
Sand Paper + water Soaking	64.00	12.67	38.33	17.00	57.00	37.00
H_2SO_4 + water Soaking	12.67	0.00	6.33	0.00	0.00	0.00
KNO_3 + water Soaking	5.33	6.00	5.67	6.00	5.00	5.50
HCl + water Soaking	13.33	12.00	12.67	5.00	4.50	15.25
HNO_3 + water Soaking	0.00	2.00	1.00	3.00	0.00	1.50
Hot Water + $GA_3$10mg/ l water Soaking	0.00	0.00	0.00	0.00	2.50	1.25
Cow dung water + Water Soaking	30.00	10.00	20.00	7.00	26.50	18.50
Cow dung Water + Gibbrellic acid	26.00	8.67	17.33	9.50	23.00	16.25
Hot Water + Water Soaking	0.00	0.00	0.00	0.00	0.50	0.25
Cow dung + Water Soaking	44.00	12.00	28.00	9.50	23.50	19.75
Control (Untreated)	34.67	0.00	17.33	10.50	30.00	4.75
CD at 5%	24.46	6.67		7.23	18.46	
Treatments	1.31			4.51		
Seed age	10.09			64.42		
Treatment x Seed age	10.34			16.29		
C.V.	40.48%			61.57%		

Table 1.2: Effect of different treatments, on seed germination of important medicinal plant species *Asparagus racemosus*

Treatments	*In vitro*			*In vivo*		
	Fresh	**1yr old**	**Mean**	**Fresh**	**1yr old**	**Mean**
Water soaking for 24 hrs	1.33	28.33	14.83	0.00	31.00	15.50
Gibbrellic acid @10,000 ppm	2.67	20.00	11.33	0.00	15.00	7.50
Sand Paper + water Soaking	0.67	13.33	7.00	0.00	17.00	8.50
H_2SO_4 + water Soaking	0.00	0.00	0.00	0.00	2.00	9.50
KNO_3 + water Soaking	0.67	28.33	14.50	0.00	34.00	17.00
HCl + water Soaking	0.00	0.00	0.00	0.00	6.00	3.00
HNO_3 + water Soaking	0.00	0.00	0.00	0.00	0.00	0.00
Hot Water + $GA_3$10mg/ l water Soaking	0.00	0.00	0.00	3.50	4.50	4.00
Cow dung water + Water Soaking	1.33	20.00	10.67	40.50	35.50	38.00
Cow dung Water + Gibbrellic acid	0.67	25.00	12.83	16.00	19.00	18.00
Hot Water + Water Soaking	0.00	0.00	0.00	46.00	47.00	46.50
Cow dung + Water Soaking	1.33	8.33	4.83	10.00	24.00	17.00
Control (Untreated)	2.67	21.67	12.67	0.00	20.00	1.00
CD at 5%	0.87	12.69		8.92	19.62	
Treatments	5.48			15.17		
Seed age	6.44			38.68		
Treatment x Seed age	13.48			54.71		
C.V.	87.75%			186.14%		

Table 1.3: Effect of different treatments on seed germination of important medicinal plant species *Abelmoschus moschatus*

Treatments	*In vitro*			*In vivo*		
	Fresh	**1yr old**	**Mean**	**Fresh**	**1yr old**	**Mean**
Water soaking for 24 hrs	52.00	32.67	42.33	86.50	31.00	58.75
Gibbrellic acid @10,000 ppm	63.33	8.00	35.67	67.50	42.50	55.00
Sand Paper + water Soaking	73.33	18.67	46.00	49.50	33.50	43.75
H_2SO_4 + water Soaking	48.00	26.67	37.33	27.00	18.00	22.50
KNO_3 + water Soaking	41.33	0.67	21.00	48.00	29.00	46.75
HCl + water Soaking	61.33	2.67	32.00	35.00	11.50	23.25
HNO_3 + water Soaking	0.00	0.00	0.00	5.00	0.00	2.50
Hot Water + $GA_3$10mg/ l water Soaking	0.00	0.00	0.00	0.00	0.00	0.00
Cow dung water + Water Soaking	50.67	10.67	30.67	55.50	0.00	27.75
Cow dung Water + Gibbrellic acid	50.00	9.33	29.67	28.50	25.50	27.00
Hot Water + Water Soaking	0.00	0.00	0.00	0.50	40.50	20.50
Cow dung + Water Soaking	47.33	17.33	32.33	56.50	18.00	37.25
Control (Untreated)	46.67	9.33	28.00	60.00	38.00	38.50
CD at 5%	41.07	10.46		39.96	22.12	
Treatments	7.08			6.98		
Seed age	19.58			17.80		
Treatment x Seed age	27.69			25.17		
C.V.	37.24%			39.37%		

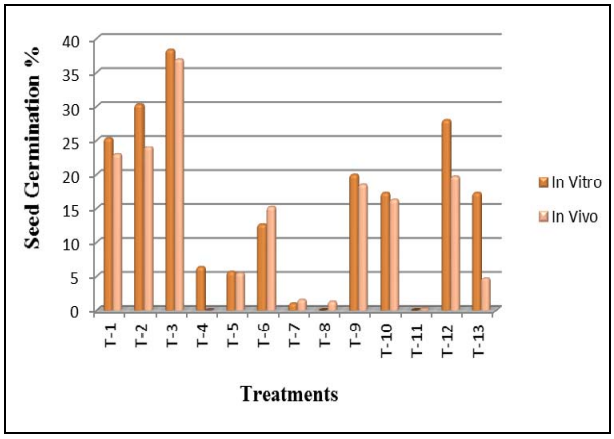

Fig 1: Mean Seed Germination in *Cassia angustifolia*.

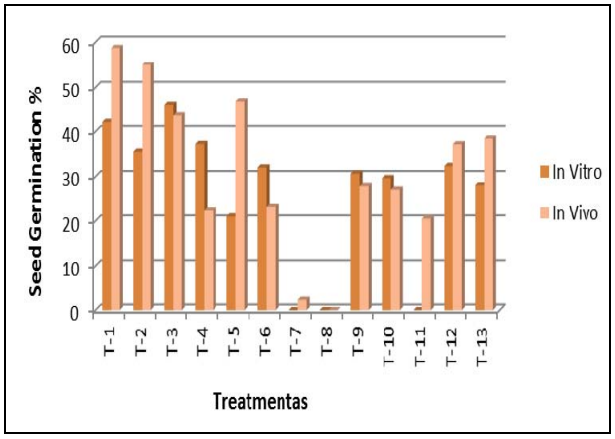

Fig 3: Mean Seed Germination in *Abelmoscus moschatus*

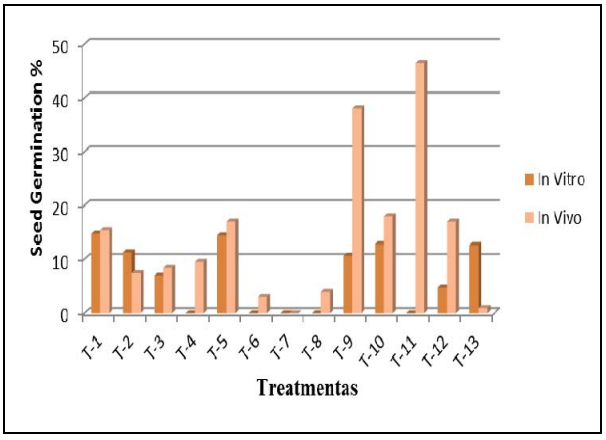

Fig 2: Mean Seed Germination in *Asparagus racemosus*

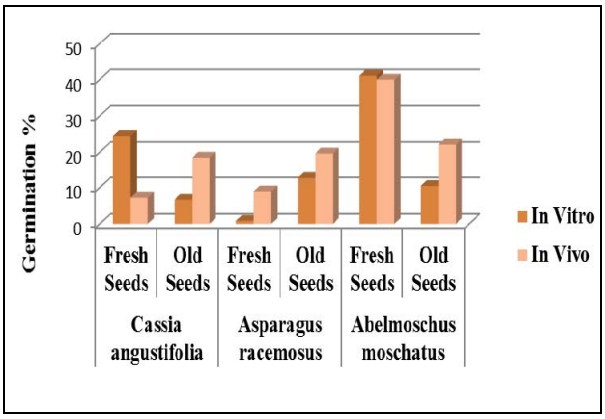

Fig 4: Comparative status of Fresh and old seeds on seed germination

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