

Techniques for Breaking Seed Dormancy and its Efficacy on Seed Germination of Six Important Medicinal Plant Species

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Paper No. 681

Received: 15-11-2017

Accepted: 16-03-2018

ABSTRACT

Present study deals the effectiveness of traditional and recent techniques of seed treatment on germination of fresh and year old seed of *Abelmoschus moschatus*, *Asparagus racemosus*, *Bixa orellana*, *Cassia angustifolia*, *Operculina turpethum* and *Psoralea corylifolia* under *in vitro* and *in vivo* conditions. Result indicates that seed treatments had significant positive impact on germination; however the effectiveness of the treatments varied among different medicinal species. There was 3 to 15 folds increment in percent germination due to seed treatments. Seed treatment with H₂SO₄, seed coat scarification by sand paper and seed soaking in normal water for 24 h rendered maximum germination, both in *in vitro* and *in vivo* conditions in most of the species under studied. Mean seed germination increased maximum 64.28%, 1460.0%, 115.40% and 274.94% in *A. moschatus*, *B. orellana*, *C. angustifolia* and *P. corylifolia* respectively over control. Seeds treated with hot water at 70°C for 1 h rendered 365% more germination in *A. racemosus* under *in vivo*, while *O. turpethum* seeds germinated maximum with H₂SO₄ 95% under both *in vitro* and *in vivo* conditions.

Highlights

- ① Scarification of seed coat with sand paper was found the most effective in breaking seed dormancy method for *A. moschatus*, *B. orellana*, *C. angustifolia* and in *P. corylifolia*.
- ② Soaking of seeds in water for 24 h proved to be best in seed germination of *A. racemosus*.
- ③ Seed treatment with H₂SO₄ (95%) + water soaking observed best in *O. turpethum*.
- ④ Freshly harvested seeds rendered higher germination in *A. moschatus* and *B. orellana* while one year old stored seeds of *A. racemosus* and *O. turpethum* germinated significantly higher.
- ⑤ Treatment of Seeds before sowing improved percent germination in all seed of medicinal valued plant species.

Keywords: Germination, *In vitro*, *In vivo*, Medicinal plant, Seed dormancy

The industrial demand for the medicinal plant resources has been on the rise due to the worldwide buoyancy in the production of herbal health care formulations; cosmetics and nutritional supplements. In India, nearly 9,500 registered herbal industries and a multitude of unregistered Vaidya depends upon the sustainable supply of raw medicinal plants (Ved and Goraya, 2007). This demand can only be full filled when such plants are to be grown in farmer's fields. Some medicinal plants viz. *Abelmoschus moschatus*, *Asparagus racemosus*, *Bixa orellana*, *Cassia angustifolia*, *Operculina*

turpethum and *Psoralea corylifolia* have growing interest among Indian farmers owing to higher income than other traditional crops as their use has been already established in curing of many diseases in human and animals since ancient time. Now the use of herb products is gaining momentum globally to avoid adverse effects of allopathic medicine.

Medicinal plants are propagated mainly through seed, which suffers dormancy (Ramamoorthy *et al.* 2005; Pallavi *et al.* 2014) using different species, while some medicinal species only germinates in their native habitat. Breaking of seed originated



dormancy is very essential for plant population and eventually yield. To break seed dormancy our fore father were used locally available methods in agriculture viz. cow urine, rubbing of seed with stones, passing of seed through animal stomach etc., are still relevant even in modern time. However, now growth regulator, acid methods, radiation techniques being replaced our indigenous traditional knowledge (ITKs). Other scientists Patane and Grestab (2006) and Kaef *et al.* (2011) has reported the beneficial effects of growth regulators and chemicals in breaking of seed dormancy in medicinal plants. Similarly mechanical scarification with sand paper (Ali *et al.* 2011), hot water (Olmez *et al.* 2008; Rahnama and Tavakkol-Afshari, 2007) and perforation of seed coat (Aliero, 2004; Sari *et al.* 2006; Heidari *et al.* 2008) have been also advocated for certain medicinal plant species. As for as literature survey could ascertain, study on seed germination was lacking on these species especially depicting both traditional and recent techniques. Therefore, the present investigation aimed to track best method in breaking dormancy of some important medicinal plants either fresh or year aged seeds under *in vitro* and *in vivo* conditions were evaluated.

MATERIALS AND METHODS

The present experiment was carried out at the Experimental Farm 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. The climate of Bilaspur is sub humid and temperature varies from a minimum of 4.6°C (in December-January) to a maximum of about 45°C (in May) with average annual rainfall 1250 mm.

Seed source: Fresh seeds of *Abelmoschus moschatus*, *Asparagus racemosus*, *Bixa orellana*, *Cassia angustifolia*, *Operculina turpethum* and *Psoralea corylifolia* were collected from medicinal plant nursery inside the college campus. One year aged seeds of the same species were also collected from the same nursery, which were stored year before in plastic box at room temperature.

Treatments: The experiment consisting of two factors viz. Seed age as first factor (fresh and one year old) and treatments as second factor under both conditions i.e. *in vitro* (Laboratory condition)

and *in vivo* (Nursery field condition). The treatments applied in the present experiment of medicinal plants is given in table 1.

Table 1: Treatment details and its combinations undertaken in the experiment.

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 ₂ SO 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)

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. In acid treatments, seeds were soaked separately in H₂SO₄ (95%), Nitric acid (HNO₃ 63.1%) and Hydrochloric acid (HCl 35%) for 1 h then washed thrice with tap water and soaked in normal distilled water further for 24 h. Gibbrellic acid (10 mg/l) and Potassium nitrate (0.2%) were used for 24 h presoaking of seeds at room temperature. For 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 while hot water followed by chemical Treatments, seeds were placed in water both at 70°C for 1 h than seed soaked in Gibbrellic acid solution (10 mg/l) for 24 h. Seed of medicinal plants were also subjected to 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. Some 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

In vitro and *in vivo* conditions, all treated seeds were sown in two factors in completely randomized design with three replications. Observations were recorded on germination. Different treatment combinations were applied to break seed dormancy using mechanical, physical and chemical methods. Fifty treated seeds along with 50 untreated seeds were kept in Petri plates having moist filter paper and 5 to 7 ml, sterilized water was further added at an interval of 24 h to provide suitable moisture for germination. Entire experiment was replicated thrice and kept *in vitro* and germination (%) was recorded after three days of incubation and continued for 18 days. *In vivo*, treated and untreated seeds (100 seeds / treatment) were sown in nursery beds as per randomized design with three replications and data was recorded after 15 days of sowing and continued up to 45 days. The data recorded was subjected to statistical analysis using SPSS software.

RESULTS AND DISCUSSION

Germination of all medicinal plants significantly influenced with seed treatments; however variation existed among species, treatments and conditions maintained for the experiments. The results data are summarized in table 2 to 4 and Fig. 1 to 3. Mean germination of *A. moschatus* found highest 58.75% with soaking of seeds in water for 24 h and kept under *in vivo* condition, while sand paper + water soaking gave highest germination 46.0% under *in vitro* compared to control seeds (Table 2 and Fig. 1a). No germination observed in seeds with Hot water +GA₃ under both *in vitro* and *in vivo* conditions. HNO₃ and hot water treated seeds also failed to germinate under *in vitro* condition. Fresh seeds were significantly more than old seeds of *A. moschatus* as mean germination was 292.63% and 80.65% higher under *in vitro* and *in vivo* conditions respectively compared to year aged seeds (Fig. 1a). In *A. moschatus* seed germination found 2.77% more under *in vitro* than *in vivo* condition in case of fresh seeds, while old seeds were germinated better under *in vivo* condition as -52.71% less germination was found under *in vitro* condition in old seeds (Fig. 3). Seed germination result of *Asparagus racemosus* is summarized in Table 2 and Fig. 1b. It was observed that the overall seed germination of the species was very poor however, Hot Water + Water Soaked

Table 2: Effect of seed treatments on germination percentage of *Abelmoschu smoschatus* and *Asparagus racemosus*

Treatments	<i>Abelmoschus moschatus</i>				<i>Asparagus racemosus</i>			
	<i>In Vitro</i>		<i>In Vivo</i>		<i>In Vitro</i>		<i>In Vivo</i>	
	Fresh	1 st old	Fresh	1 st old	Fresh	1 st old	Fresh	1 st old
T-1	52.00	32.67	86.50	31.00	1.33	28.33	0.00	31.00
T-2	63.33	8.00	67.50	42.50	2.67	20.00	0.00	15.00
T-3	73.33	18.67	49.50	33.50	0.67	13.33	0.00	17.00
T-4	48.00	26.67	27.00	18.00	0.00	0.00	0.00	2.00
T-5	41.33	0.67	48.00	29.00	0.67	28.33	0.00	34.00
T-6	61.33	2.67	35.00	11.50	0.00	0.00	0.00	6.00
T-7	0.00	0.00	5.00	0.00	0.00	0.00	0.00	0.00
T-8	0.00	0.00	0.00	0.00	0.00	0.00	3.50	4.50
T-9	50.67	10.67	55.50	0.00	1.33	20.00	40.50	35.50
T-10	50.00	9.33	28.50	25.50	0.67	25.00	16.00	19.00
T-11	0.00	0.00	0.50	40.50	0.00	0.00	46.00	47.00
T-12	47.33	17.33	56.50	18.00	1.33	8.33	10.00	24.00
T-13	46.67	9.33	60.00	38.00	2.67	21.67	0.00	20.00
CD (5%) T*	7.08		6.98		5.48		15.17	
A	19.58		17.80		6.44		38.68	
T X A	27.69		25.17		13.48		54.71	

*T- Treatment, A-Seed age, T X A – Treatment X Seed age

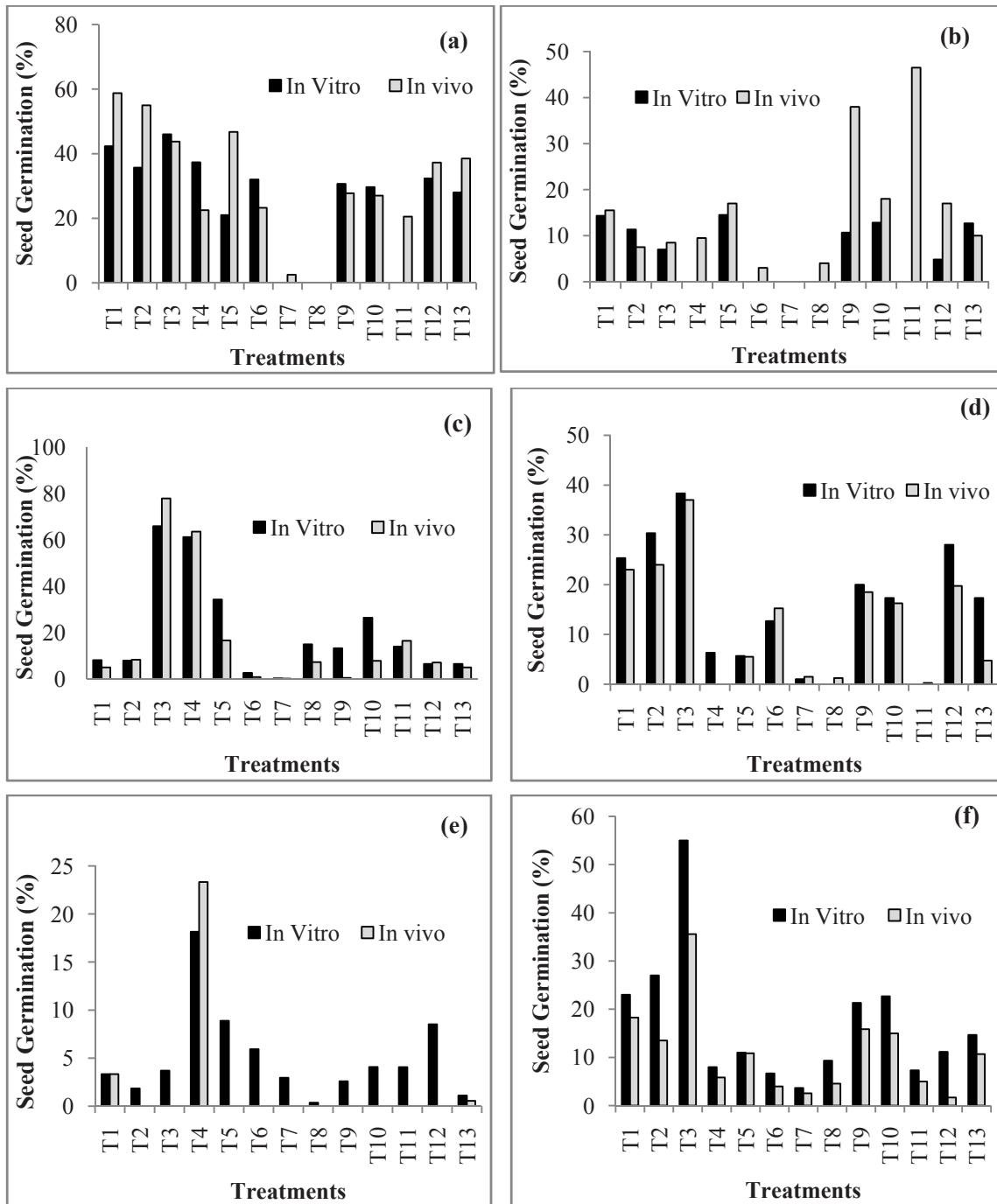


Fig. 1: Mean seed germination (%) after different seed treatment in (a) *A. moschatus* (b) *A. racemosus* (c) *Bixa orellana* (d) *C. angustifolia* (e) *O. turpenthum* (f) *P. corylifolia*

seeds germinated highest 46.50% and Cow dung water + Water Soaked seed germinated 38.0% were the most effective treatment under *in vivo* as compared to control seeds and water soaked seeds for 24 h rendered highest mean germination 14.83% under *in vivo* compared to control seeds which recorded 12.67% and 10.0% germination under *in vitro* and *in vivo* conditions respectively (Table 2).

In *A. racemosus*, fresh seeds germinated poorly than year aged seeds (mean germination 0.87 and 8.92% in fresh seeds and 12.69 and 19.62% in old seeds respectively (Fig. 2)). In addition, *in vivo* condition was found to be more suitable in seed germination than *in vitro* condition when compared with their respective categories (Fig. 3).

Bixa orellana seeds treated with different physical and chemical methods are given in Table 3 and Fig. 1c. It indicates that the highest 92.33% germination found in seeds treated with H₂SO₄ + water soaking under *in vivo* and 75.33% with sand paper + water soaking under *in vitro* condition. Maximum seed germination in control seeds found 13.33% under *in vitro* and 10% under *in vivo* condition, which depicts the positive efficacy of seed treatments in enhancing seed germination (Table 3).

Mean seed germination was maximum 78% with sand paper + water soaking under *in vivo* and 66% with the same treatments under *in vitro* condition (Fig. 1c). It was observed that the fresh seeds germination 60.14% and 12.16% higher than the germination in aged seeds under *in vitro* and *in vivo* condition respectively (Fig. 2). Overall, fresh seeds germinated 41.22% higher under *in vitro* condition than *in vivo*, while no change in the germination of old seeds under both the conditions (Fig. 3).

Seed germination in *C. angustifolia* was found very poor germination even after using various seed treatment techniques, however only few treatments exhibited positive results (Table 3). Mean germination was enhanced from 17.33 of control to 38.33% in seeds treated with sand paper + water soaking under *in vitro* and similarly 20.25%

to 37.0% under *in vivo* with the same techniques which was found 115.40% and 678.94% higher mean germination than its controls. (Fig. 1d). The germination of fresh seed was 266.71% higher than old seeds under *in vitro* while old seeds germinated better under *in vivo* condition (Fig. 2).

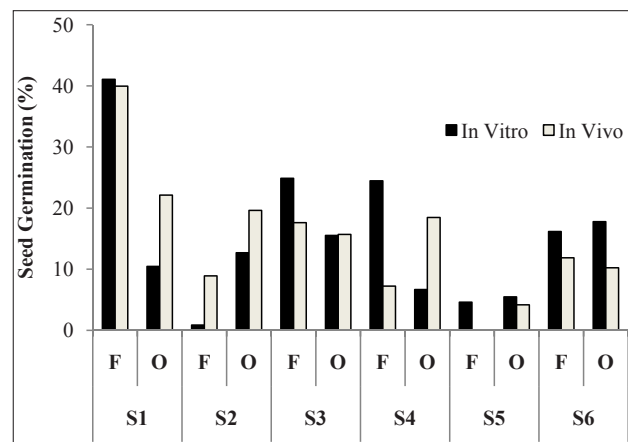


Fig. 2: Mean Seed Germination (%) in fresh seed (F) and old seeds (O) of (s1) *A. moschatus* (s2) *A. racemosus* (s3) *B. orellana* (s4) *C. angustifolia* (s5) *O. turpethum* and (s6) *P. corylifolia*

Some other treatments viz. Gibbrellic acid and water soaking of seeds for 24 hrs also found to be beneficial in breaking seed dormancy as compared to control seeds. The mean germination of fresh seeds was 238.32% higher under *in vitro* than

Table 3: Effect of seed treatments on germination percentage of *Bixa orellana* and *Cassia angustifolia*

Treatments	<i>Bixa orellana</i>				<i>Cassia angustifolia</i>			
	<i>In Vitro</i>		<i>In Vivo</i>		<i>In Vitro</i>		<i>In Vivo</i>	
	Fresh	1 ^{yr} old	Fresh	1 ^{yr} old	Fresh	1 ^{yr} old	Fresh	1 ^{yr} old
T-1	16.33	0.00	10.00	0.00	39.33	11.33	13.50	32.50
T-2	15.67	0.33	14.66	2.00	48.67	12.00	13.00	35.00
T-3	75.33	56.67	91.00	65.00	64.00	12.67	17.00	57.00
T-4	48.00	74.67	35.00	92.33	12.67	0.00	0.00	0.00
T-5	68.33	0.33	32.67	0.66	5.33	6.00	6.00	5.00
T-6	0.00	5.33	0.00	1.67	13.33	12.00	5.00	4.50
T-7	0.00	0.67	0.00	0.33	0.00	2.00	3.00	0.00
T-8	1.00	29.00	01.33	13.33	0.00	0.00	0.00	2.50
T-9	21.00	5.67	0.33	0.67	30.00	10.00	7.00	26.50
T-10	50.33	2.67	15.00	0.67	26.00	8.67	9.50	23.00
T-11	1.33	26.67	21.00	12.00	0.00	0.00	0.00	0.50
T-12	13.00	0.00	14.00	0.33	44.00	12.00	9.50	23.50
T-13	13.33	0.00	10.00	0.00	34.67	0.00	10.50	30.00
CD (5%) T	3.43		3.70		1.31		4.51	
A	9.62		4.58		10.09		64.42	
T X A	9.86		13.36		10.34		16.29	

*T- Treatment, A-Seed age, T X A – Treatment X Seed age

germination of fresh seeds under *in vivo*, while year aged seed performed higher germinated under *in vivo* condition (Fig. 3).

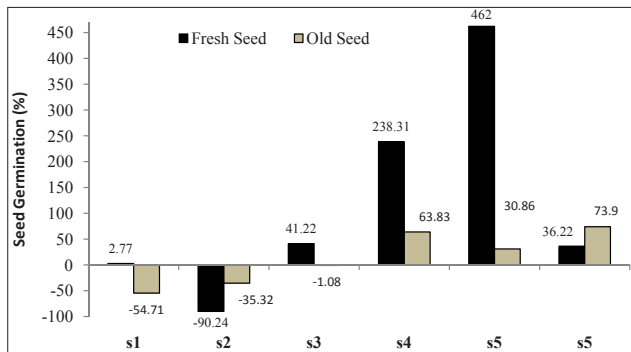


Fig. 3: Mean Seed Germination (%) of (s1) *A. moschatus* (s2) *A. racemosus* (s3) *B. orellana* (s4) *C. angustifolia* (s5) *O. turpethum* and (s6) *P. corylifolia* seeds when compared with *in vitro* and *in vivo* conditions in its respective fresh and old seeds

The germination of *Operculina turpethum* seed was found to be extremely low in most of the treatments under *in vivo* condition even after treatments. However, under *in vivo* condition, H_2SO_4 water soaking was found highly effective in improving the mean germination by 41.48 folds (*in vivo*) and 15.35 folds (*in vitro*) over control seeds. The highest germination recorded 46.66% in year aged seeds under *in vivo* and 26.67% in fresh seeds under *in vitro* in H_2SO_4 water soaking treated seeds (Table 4), while mean germination in control seed was found 1.11% (*in vitro*) and 0.50% (*in vivo*) (Fig. 1e). It was also observed that fresh seeds germination was -15.53% poor than old seeds under *in vitro* while no fresh seeds were germinated under *in vivo* (Fig. 2), this indicated the superiority of old seeds as compared to fresh *O. turpethum* seeds. Seeds grown under *in vitro* condition rendered 462% and 30.86% higher germination in fresh seed and old seeds respectively in comparison to seed germination under *in vivo* condition (Fig. 3).

In *Psoralea corylifolia*, different seed treatments had significant positive effect on germination as compared to untreated seed (Table 4). Seed coat scarification with sand paper + water soaking was found to be most effective and showed significantly highest mean germination (55.0%) under *in vitro* and under *in vivo* (35.57%) conditions (Fig. 1f), these were 274.91% and 232.42% higher over germination in control seeds. Gibbrellic acid, Cow dung + water soaking and water soaking for 24

h also found to induce germination significantly than other treatments. Mean germination in fresh seeds was -9.10% poor than aged seeds under *in vitro*, but fresh seeds germinated 16.03 % higher than old seeds under *in vivo* (Fig. 2). Comparing the seed germination between *in vitro* and *in vivo* condition, germination under *in vitro* was superior as mean germination under *in vitro* was 36.22% (fresh seed) and 73.90% (old seed) higher compared to germination under *in vivo* condition (Fig. 3).

Present study justifies the significance of different treatments in breaking seed dormancy and enhancing seed germination in all six medicinal plants. However, the effectiveness of treatments varied in different species which indicates the needs of specific treatment for each species. Amongst 13 treatments, few of them significantly enhance the germination, while others were found ineffective compared with untreated control seeds. This indicates the necessity of seed treatment with their integrated combinations before sowing.

Sand paper followed by water soaking was found most effective for *B. orellana*, *C. angustifolia* and *P. corylifolia* both under *in vitro* and *in vivo* conditions compared to other treatments. This indicates the significance of rupturing seed coat surface to allow, water and oxygen readily to the embryo resulted into higher germination (Cavanagh 1980). Baskin and Baskin (2004) have also reported seed coat dormancy in 16 families of angiosperm causes delayed or no germination. Poor germination of control seed might be due to the presence of this reason. Several other workers have propounded sand paper scarification as one of the effective method in breaking dormancy in many plant species (Kaef *et al.* 2011; Ali *et al.* 2011; Kildisheva *et al.* 2011; Venier *et al.* 2012; Zare *et al.* 2011).

Seed underwent soaking in water for 24 h too rendered highest mean germination in *A. moschatus*, *A. racemosus* and *P. corylifolia* while, second most effective treatments in *C. angustifolia* and in *O. turpethum*. This might be due to softening of seed surface and rapid imbibitions of embryo as also reported (Xu *et al.* 2004; Asi *et al.* 2011). They have reported that the higher amount of water penetration into seed not only imbibe embryo but also helps in releasing of simple sugar which utilized in protein synthesis. In addition, release of auxin and ethylene may also facilitate seed germination through

Table 4: Effect of seed treatments on germination percentage of *Operculina turpethum* and *Psoralea corylifolia*

Treatments	<i>Operculina turpethum</i>				<i>Psoralea corylifolia</i>			
	<i>In Vitro</i>		<i>In Vivo</i>		<i>In Vitro</i>		<i>In Vivo</i>	
	Fresh	1 st old	Fresh	1 st old	Fresh	1 st old	Fresh	1 st old
T-1	2.22	4.45	0.00	6.67	26.00	20.00	19.85	16.57
T-2	0.00	3.70	0.00	0.00	33.33	20.00	21.14	6.57
T-3	6.67	0.74	0.00	0.00	56.00	54.00	30.28	40.85
T-4	26.67	9.63	0.00	46.66	9.33	6.67	7.71	3.99
T-5	4.44	13.33	0.00	0.00	10.67	11.33	10.28	11.42
T-6	4.44	9.63	0.00	0.00	2.667	10.67	3.99	3.99
T-7	2.22	3.70	0.00	0.00	0.00	7.33	0.57	4.57
T-8	0.00	0.74	0.00	0.00	2.667	16.00	0.57	8.57
T-9	2.22	2.96	0.00	0.00	20.00	22.67	24.28	7.42
T-10	4.44	3.70	0.00	0.00	18.00	27.33	8.85	21.14
T-11	6.67	1.48	0.00	0.00	2.00	12.67	2.57	7.42
T-12	2.22	14.82	0.00	0.00	13.00	9.33	1.67	0.83
T-13	0.00	2.22	0.00	1.11	16.67	12.67	16.57	4.85
CD (5%) T	23.69		2.11		4.56		0.80	
A	25.27		5.38		3.33		4.24	
T X A	21.11		7.61		13.07		4.89	

*T- Treatment, A-Seed age, T X A – Treatment X Seed age

increase in nucleic acid metabolism and protein synthesis (Jackson 1994).

Acid scarification techniques have been already proved beneficial for breaking seed dormancy and enhancing germination by many workers (Levit 1974; Sovler and Khawar 2006). In *O. turpethum* H₂SO₄ (95%) followed with water soaking was found most effective while other treatments were ineffective. *B. orellana* was also influenced with the same acid also concord with other findings (Levit 1974; Youssef 2008) as the concentrated H₂SO₄ also disrupts the seed coat and improves germination. Actually, this acid is thought to disrupt the seed coat and expose the lumens of the macrosclereids cells, permitting imbibitions of water (Nikoleave 1977) which triggers germination as in case of *Prosopis koelziana* and *P. juliflora* (Sovler and Khawar 2006), *Parkia biglobosa* (Aliero 2004) and *Cassia fistula* (Kandya 1999; Al-Menaie *et al.* 2010). KNO₃ 0.2% for 24 h was also effective in *A. racemosus* and *O. turpethum* might possibly through oxidized forms of nitrogen causing a shift in respiratory metabolism to the pentose phosphate pathway (Robert and Smith 1977).

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. The contact of seeds with hot water rupture the coat wall allowing water to permeate the seed tissues causing physiological changes results rapid germination of the embryo (Sabongari 2001; Rincon *et al.* 2003). The adverse effects of hot water were also observed in present study except in *A. racemosus* (*in vivo*), *B. orellana* (*in vitro*) and *O. turpethum* (*in vitro*) might be because of the long duration contact of seed with hot water results damage of embryo. Similar result was also reported in *A. africana* when seeds were treated with 100°C hot water for 12 h (Amusa 2011).

GA3 a growth promoting hormone acts as stimulant for embryos also confirmed our findings in *A. moschatus* (*in vivo*) and *P. corylifolia* (*in vitro*) as similar to results propounded by several researchers (Zhang and Maun 1990; Chen *et al.* 2005; Tzortzakis 2009). Pre sowing treatment with GA3 under *in vivo* condition stimulated seed germination in *A. moschatus* might be due to altered physiology of embryos and activation of enzymes (Kattimani *et al.* 1999; Salisbury and Ross 2000).

Fresh seeds of *A. moschatus*, *B. orellana*, *C. angustifolia* (*in vitro*), *P. corylifolia* (*in vivo*) 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), 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 two species *A. racemosus* and *O. turpethum* showed higher germination employing aged seeds, probably due to undeveloped embryo, which required some time to be mature and have extra advantages, that their seeds can be stored for longer period of time maintaining viability (Zhang and Maun 1999). Similarly variations on germination existed between *in vitro* and *in vivo* condition of the experiment among species and age of the seedlings.

CONCLUSION

Understanding germination requirements of medicinal plant species is one of the most important steps in the survival of these species. Findings of our study revealed that seed dormancy of all medicinal species under study is caused by hard and water impermeable seed coat. A high level of germination was observed by using traditional scarification technique of seed coat and making it permeable to water and oxygen through various methods. It was found that mechanical scarification with sand paper was the most effective dormancy breaking methods for *A. moschatus*, *B. orellana*, *C. angustifolia* and in *P. corylifolia* seeds. Soaking of seed in water for 24 h was also proved best for *A. racemosus* and H_2SO_4 (95%) + water soaking for *O. turpethum*. Based on the results it may be concluded that each species requires specific methods of seed treatments depending on the types of dormancy, however, scarification of hard seed coat using H_2SO_4 or sand paper may be applied.

In general old indigenous knowledge of seed priming by soaking of seeds in water for 12 h may also be advisable for higher seed germination medicinal seeds.

ACKNOWLEDGEMENTS

A financial support was gratefully received from the Chhattisgarh Council of Science and Technology, Raipur, C.G., India.

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