

Evaluation of Sulfuric Acid Application in Breaking Dormancy of *Sida cordifolia* Seeds — An Ayurvedic Medicinal Plant

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ABSTRACT: 'Bala' (*Sida cordifolia* L. Malvaceae) is an important ayurvedic medicinal plant used in the treatment of rheumatism, asthma, bronchitis, neuralgia, sexual debility etc. An experiment was conducted to evaluate the effect of different concentration and treatment duration of sulfuric acid on breaking the seed dormancy in *Sida cordifolia*, for optimizing its germination potential. The treatments included pretreatment of seeds with 50 and 100% sulfuric acid for 5, 15 and 25 minutes. The results showed that with the increase in the duration of treatment with sulfuric acid (50%), the seed germination percentage increased from 2% to 22% and radicle length and dry weight also increased from 3.543 to 6.067 cm and from 0.6 to 0.683mg, respectively. There was no significant difference among the treatments with sulfuric acid 100 % for 5, 15 and 25 minutes.

Key words: Sulfuric acid; *Sida cordifolia*; Seed dormancy breaking

'Bala' (*Sida cordifolia* L. Malvaceae), commonly known as Indian ephedra due to presence of ephedrine, pseudoephedrine, etc. is an important ayurvedic medicinal plant used in the treatment of rheumatism, asthma, bronchitis, neuralgia and sexual debility [1-2]. Seed coat impermeability to water is an important factor in the dormancy of seeds of *Sida* [3]. The seed of *Sida* is extremely hard due to impermeable seed coat that prevents the embryo from imbibing water, thus impairing seed germination [4, 5], which indicates physical dormancy or hard seededness [6, 4]. Physical dormancy in *Sida* species is caused by presence of water impermeable palisade cell layer in seed coat, which is impregnated with suberin, cutin and lignin like hydrophobic substances. Scarification of *Sida* seed through chemical treatments such as sulfuric acid, for breaking physical dormancy is required before sowing, to enhance seed germination. The treatment ruptures the palisade cell layer thereby improving the seed coat permeability [7, 8, 9].

Preliminary experiments showed that the

seeds of *Sida cordifolia* were not able to germinate under normal conditions. According to Seal and Gupta [10], the temperature of 25-35°C was regarded as optimum for high seed germination. Pretreatments such as cold treatment, leaching, seed coat puncturing and conc. H₂SO₄ treatments were given to enhance the percentage of germination. Pretreatment with conc. H₂SO₄ showed significant result to break the dormancy in *Sida cordifolia* [11]. Keeping these in background the present investigation was done to elucidate the optimum concentration and duration of sulfuric acid to break dormancy to achieve maximum seed germination for getting uniform quality seedlings for successful commercial cultivation.

The seeds of *Sida cordifolia* were collected from research farm of ICAR-DMAPR, Anand, Gujarat during Autumn, 2015. To maximize precision and minimize errors, seeds with uniform size and color were selected. The study was carried out at the Directorate of Medicinal and Aromatic Plants Research (DMAPR), Boriavi,

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Anand, Gujarat in 2015 (72° 55' E longitude and at an altitude of about 45.1 MSL) using a Complete Randomized Design with three replications. Based on the information available in the literature, two concentrations viz. 50% and 100% H₂SO₄ were selected for the present study. The treatments included placing the seeds in sulfuric acid (50 and 100 %) for 5, 15 and 25 minutes. Then, the seeds were thoroughly washed with distilled water for 5 minutes, and placed in 9-cm-diameter petri dishes at the rate of one hundred seeds per dish on two layers of filter paper (Whatman Grade No. 1) and one was used to cover the seed. Each Petri dish was supplied with 5 ml distilled water and 0.5 ml fungicide (Benomyl 2:1000) to prevent fungal growth. The dishes were placed in incubator set to 35°C under darkness. After 72 hours, the germinated seeds were counted. This was continued until the maximum germination was obtained (15 days). At the end, germination percent, seedling length radicle length and seedling dry weight were calculated for each treatment/replication [12].

From each replication, five normal seedlings were taken randomly to measure the mean seedling length (root + shoot length) and mean radicle length (cm).

The same five seedlings were placed in a paper envelop and dried initially in shade for 16 hrs, followed by drying in oven set at 75°C± 5°C for 48 hrs after which they were weighed to determine average seedling dry mass. Data were subjected to analysis of variance (ANOVA) and mean separation. Using GenStat® Release 15.1 and Excel, respectively. The means were compared by LSD test at 5% probability level.

Analysis of variance revealed that the concentration of sulfuric acid was highly significant for germination percentage, radicle length and radicle dryweight (Table 1). The germination per cent ranged from 4.33 to 22% in treatments as compared to 2% in control. When seeds were put in lower concentrations of sulfuric acid for a longer period, the germination percentage significantly increased and the maximum germination of 22% was recorded at pretreatment with 50% sulfuric acid for 25 minutes. However, it was reverse, i.e. the increase in duration of treatment with 100% H₂SO₄ significantly reduced the germination percentage which might be due to the death of embryo [13] as already reported in *Zanthoxylum armatum* [14] where scarification with 50% sulfuric acid for 15 minutes was more effective. Disintegration of the seed coat as well as micropylar plug do facilitate subsequent imbibition and germination of seeds treated with concentrated sulfuric acid [15]. The difference in percent germination in different studies with same or different concentration and treatment duration of sulfuric acid is expected, as every species has its own makeup of seed structure. Duration of sulfuric treatment also showed significant results in *Canna indica* for germination and seedling length and weight [8]. However, in the present study, the maximum germination percent recorded (i.e., 22%) with sulfuric acid treatment, as compared to control (2%), is comparatively lower than other studies, indicating that still there is a scope to further increase the germination per cent through breaking the seed dormancy by altering the duration and concentration of sulfuric acid or combination of both. For example, the treatment

Table 1. Analysis of variance for different periods of treatment with different sulfuric acid concentrations in seeds of *Sida cordifolia*

Source of variation	df	Means of squares		
		Germination percentage	Seedling	Seedling dry weight
Replication	2	0.619	0.027	0.0000417
Treatment	6	153.968*	7.100**	0.0260640**
Error	12	2.563	0.015	0.000048

**Significant at P = < 0.01; *P= <0.05, respectively

at 50% sulfuric acid for 15 minutes and 100% acid for 5 minutes gave almost same germination percent (statistically, at par). Hence, it appears that the lower concentration treatment should be given for longer time whereas the higher concentration of acid should be given for shorter time. Optimizing the dose and duration is a crucial factor for each and every species.

The seedling length also significantly different among the seven treatments (Table 1). In the lower concentration, lowest radicle length (3.543 cm) was observed in 5 minutes pretreatment with sulfuric acid and the highest one was observed in 25 minute pretreatments with sulfuric acid (6.067 cm). But in the higher concentration (100%), length of the radicle was higher (4.01 cm) in 5 minutes treatments and lower (1.077 cm) in 15 minutes treatment.

For radicle dry weight also, significant difference among treatment was observed (Table 1). Radicle dry weight increased from 0.6 mg at 5 minutes pretreatment with sulfuric acid to 0.667 and 0.683 mg at 15 and 25 minutes, respectively. While with the increase of sulfuric acid concentration (100%), the seedling dry weight decreased subsequently with the duration of pretreatments for 5, 15 and 25 minutes and the weight of radicles were 0.5127, 0.504 and 0.427 mg, respectively which were very less i.e. implying poor survivability. Nosrati *et al.* [16] showed that the most effective method for breaking the dormancy of seeds of *Atriplex canescens* was putting the seeds in sulfuric acid for 30 minutes which produced the highest germination percentage and seedling weight. But treating the seeds of *A. halimus* with sulphuric acid for 10, 20, 30 and 60 minutes were not an appropriate way for breaking their dormancy because the embryos were damaged which is consistent with the results of the current study.

Since the sulfuric acid treatment (50%) increased the germination percentage by breaking the seed dormancy to some extent, it was suggested that type of dormancy present in *Sida cordifolia* may be morpho-physiological. By taking the lead from study, different combinations of treatments may be applied to break dormancy for maximum germination and survival

percentage.

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