Assessment of Compatible Substratum for *Andrographis paniculata* Standard Seed Germination Testing

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Abstract:

Andrographis paniculata (Burm. f.) Wallich *ex* Nees contains a bitter compound called andrographolide is commonly used for medicinal purposes. The herb has been revered for treating infectious diseases and highly regarded also as having a preventative effect from many diseases, due to its powerful immune strengthening benefits. The propagation of *A. paniculata* through seed is somewhat difficult which may be due to various germination problems. In view of the above, the present investigation was conducted with an objective to assess the compatible substratum for standard seed germination test of *Andrographis* seed. The seeds were collected and stored for six months at optimum temperature. Four substrata namely filter paper, top soil, saw dust and riverbed sand was collected for the experiment. Sand and soil were sieved with a 2 mm sieve to ensure uniform particle size. A standard germination test was done under partly sterilised and non-sterilised substratum with proper maintenance of light (11 hrs) and temperature (25^0C) by adding water up to 60 –70 % WHC. The 16 days test showed varied germination index and number of secondary roots. The result showed that the sterilized filter paper has proved most effective and compatible substratum for the *Andrographis paniculata* standard seed germination test. This led to uniform and quick germination with proper secondary roots and primary leaves. It can be suggested that due to scarcity of filter papers, sand substratum can also be used as an alternate as it showed more uniform germination than the rest two substrata. [The Journal of American Science. 2009; 5(6): 70-75]. (ISSN 1545-1003).

Key words: Substratum, Sterilized, Non-sterilized, *Andrographis paniculata*, Germination

Introduction

Andrographis paniculata contains a bitter compound called andrographolide; (alkaloid of pharmaceutical importance) is commonly used for medicinal purposes. *Andrographis* also called King of Bitters, is a traditional Chinese, Southeast Asian and Indian herb, and used for centuries in Ayurvedic medicine. The herb has been revered for treating infectious diseases and highly regarded also as having a preventative effect from many diseases, due to its powerful immune strengthening benefits. The demand of *Andrographis paniculata* is increasing day by day due to its importance in the treatment of different ailments.

Seeds are fundamental to agriculture and natural ecosystem. Production of high quality seeds is fundamental to the success of agriculture. Every farmer is sensitive to need for the rapid uniform seedling emergence and establishment of an even and productive stand. Crop production relies heavily on high quality planting seeds. Therefore, Government of India enacted Seeds Act so that seed sold conform to the minimum limits of physical and genetic purity, germination, moisture content and seed health. These seed quality parameters (known as Seed Standards) have been notified for more than 95 crops viz. cereals, pulses, vegetables etc. However, no such standards of seed quality parameters are available for medicinal crops. Seed testing protocols are also not available, which is a pre requisite for testing the seeds and also for recommending minimum limits of

germination (Parihar, 2006; Parihar and Kumar, 2006). Seed testing protocols are regularly updater by ISTA (International Seed Testing Association) on the basis of research work done globally through publication of research papers. The latest ISTA rules (ISTA, 2008) contain seed testing protocols of a large number of species cultivated all over the world and it forms the basic reference book for all kinds of seed testing activities and also for the international seed trade. As the seed trade developed between countries, seed testing played an important role in guaranteeing good quality seed to the farmer (Gassim, 1988).

It has become mandatory to standardise the seed testing and cultivation practices. In view of the above, studies have been carried out on germination ecology of *A. paniculata* locally known as *'Kalmegh'* and widely used in India in the treatment of the various ailments, in order to formulate seed testing protocols by obtaining information on seed germination, dormancy status and mechanism, and seed moisture relation for storage etc. The propagation of *A. paniculata* generally occurs through seeds, although it has many germination problems. The plant grows wild in tropical, moist and deciduous forests and widely cultivated in southern Asia, where it is used as dried of fresh leaves or the aerial portions of the plant to treat infections. Kalmegh can be grown on a variety of soil. In the natural habitat, it is found growing in clay to sandy soil in various locations. However, sandy loamy soil rich in organic matter is good for its growth and yield (Farooqi and Sreeramu, 2001). Saraswathy *et al*., (2006) have studied the seed ecological aspects on Kalmegh and reported problem in seed germination. It was assumed that seeds of Kalmegh possessed the combined dormancy of physical and innate nature. Among substrata that can be used for seed testing are paper towel, kimpack, blotter paper, filter paper, cotton wool, sawdust, and soil (Muliokela and Kaliangile, 1995; Louwaars and Marrewijk, 1996). Paper towels and sand are the most commonly used for testing seed of grain crops. Skinner and Schroeder (1978) reported that in soybean rolled towels gave more uniform results than sand. Thus the present study was undertaken with an objective to assess compatible substratum for standard seed germination test of *Andrographis paniculata*.

Materials and Methods

The present investigation was conducted at the Seed Testing Laboratory, Department of Seed Science and Technology, HNB Garhwal Central University, Srinagar Uttarakhand, India. The seeds were collected from Sushila Tiwari Herbal Garden, Rishikesh, Uttarakhand, India and stored for six months at optimum temperature. The experiment was laid out in a Randomized Block Design (RBD) with 4 replications for each substratum.

Seeds require certain conditions for normal germination. The most important requirements are substrata, moisture, temperature and light. The substrata serve as a moisture reservoir and provide a surface or medium for which the seeds can germinate and the seedlings grow. The commonly used substrates are paper, sand and soil. Most widely used paper substrates are filter paper; these are easy to handle versatile and comparatively cheap. To use as substrata for germination testing, different substratum namely filter paper, top soil, saw-dust and riverbed sand were collected. Sand and soil were sieved with a 2 mm sieve to ensure uniform particle size. The standard germination test was done using partly sterilised (by using autoclave) and non-sterilised substratum with proper maintenance of light (11 hrs) and temperature (25^0C). The four substrata were separately mixed with water up to 60 –70% water holding capacity (WHC). Each substratum were replicated four times and every replication contains twenty five seeds which evenly-spaced on the top of substratum and covered with the substratum up to about 2 cm level. This treatment is eco-friendly and cheaper than the other seed treatments.

Seed germination was recorded from the 3rd days after sowing (DAS) up to 13 days consecutively and germination index (G.I.) was computed by using the formula: $G.I. = \sum n/d$

Where, n =number of seedlings emerging
d = days after planting

Germination percent was calculated based on the final recorded germination value of each replicate and mean was computed. After final emergence, 5 seedlings from each replicate were selected randomly to record the number of secondary roots and primary leaves and pooled data were subjected

to analysis of coefficient of variability and means were compared using least significant difference (LSD) test at 0.05 probability level (Steel and Torrie, 1984).

Results and Discussion

The highest germination index was observed on sand substratum (non-sterilized condition) followed by soil on the same condition and lowest was recorded in saw-dust substratum of sterilized condition (Fig.1). But when the GI variation was considered between the two substrata conditions, significant and insignificant variation was found in soil and saw dust, respectively. Rapid and uniform emergence is essential for optimum field emergence and plant stand under all environmental conditions and especially under sub-optimum conditions. In the natural habitat, Kalmegh is found growing in clay to sandy soil in various locations. However, sandy loamy soil rich in organic matter is good for its growth and yield (Farooqi and Sreeramu, 2001). In the present study the germination index recorded maximum in sand media of non-sterilized condition followed by soil, filter paper and saw-dust media. In all the four substrata significant variation was found between sterilized and non-sterilized conditions except filter paper substratum test (Table 1). It is evident from the recorded observations that there was no profound effect of sterilized or non-sterilized media if the seeds have good quality. This may be the reason that either germination index or germination percent, the maximum values was recorded in non-sterilized condition. However, ISTA has recommended sterilized media for standard seed germination test under optimum condition. Saraswathy, et al., (2006) have also conducted the similar study aimed to standardize the procedure for the evaluation of germination and vigour of true seeds and to identify suitable substratum.

Seed germination test under different temperature and substrate conditions was conducted by De Almeida et al., (2009) in which they reported good performance on seed germination using the optimum temperatures with paper substrates. Renata Aparecida et al., (2004) found that the substrates apparently allowed the best combinations of water and oxygen availability for the seeds. Seed germination can take place in any kind of substrate as long as it allows for a good aeration. Importance of good substrate as an important factor for fig tree seed germination have been justified by the findings of Shuling Lin, *et al*., (2008). Gunilla Oleskog and Kenneth Sahlén. (2000) have compared moisture conditions and Scots pine (*Pinus sylvestris* L.) seed germination percentages in four types of seedbed substrates and suggested that the preparation should enhance not only the substrate's water-holding capacity, but its thermal conductivity as well. Fitch, Elizabeth A. *et al*., (2007) have carried out the similar seed germination testing and suggested that seeds of the rare annuals *Lesquerella perforata* Rollins and *L. Stonensis* rollins (Brassicaceae) germinated to higher percentages on topsoil and filter paper than on masonry sand and clay sand. In addition, mucilage production was consistently less on topsoil than on the 2 types of sands. An increase in mucilage thickness was correlated with a reduction in germination. They recommended germinating seeds of both Lesquerella species on topsoil or filter paper for best results. Effects of seed age, germination substrate, gibberellic acid, light, and temperature on seed germination on *Flourensia cernua* (Asteraceae) have been conducted by Valencia-Diaz et al., (2003) and investigated whether low germination was due to moisture conditions provided by the germination substrate, or specific conditions of light and temperature. Germination was higher on an agar substrate than on a substrate of filter paper with cotton suggesting the advantage of a constant moisture condition. They suggested that a reduced germination might have been caused by androgamic depression and not caused by the substratum.

The germination percent was recorded maximum in sterilised condition of sand substratum followed by soil substratum. The germination percent obtained on soil and filter paper was comparable. Although germination percent was high on soil and insignificant difference with sand, a high variation was found (Table-1) and the use of soil substratum in seed testing may mislead the result and this was not in accordance with the ISTA's (1987) recommendation. But least variation was observed between sterilised and non-sterilised condition in the sand and filter paper substrata which showed a uniform germination irrespective of sterilised or non-sterilized. During experimentation the saw-dust media took a long mean germination period with low germination percent which revealed that saw dust substratum was not suitable for standard seed germination test of *Andrographis*. In the recorded data of the present investigation, although sand

substratum was found best for seed germination but filter paper or paper towel is suggested as it is easy to handle and use, cheap, non-toxic, and free from pathogens. However, sometime filter paper or towel paper may not readily be available and often costly in many developing countries (Louwaars and Marrewijk, 1996); sand substratum can be used as it is cheaper, readily available, easy-to-use substratum and are comparable to internationally acceptable standards with uniform results. This substratum can also provide uniform moisture for germination in seed testing and also re-usable by sterilizing. The highest secondary root development was recorded on filter paper substratum (sterilised condition) followed by sand on the same condition. In all the substrata tests, the recorded values of primary leaves were observed similar to secondary leaves, so, coefficient of variation and standard error was found zero (0) as there was no variation in the result. Thus the higher contribution of unaccountable sources of variation (lumped together as error) to the mean of germination and germination index and other traits indicate that many other extraneous factors (for example light, humidity/moisture and temperature conditions) need to be considered in seed testing. The recommendation that seed testing should be done under controlled conditions (temperature, light, humidity, etc.) could also be explained against this background. Similarly, the magnitude of the contributions of the known sources of variation to the means for seedling traits indicate that the choice of substratum should receive a higher priority when seed testing involves seedling evaluation. A combination of suitable substratum that will permit satisfactory expression of relevant seedling traits and a high degree of control of atmospheric condition to a level with minimal fluctuation is required for seed testing.

After considering all the pooled and analysed data, of the four substrata used in this study, sterilised sand followed by unsterilized have given the best results. The results herein reported seem also to indicate that the substrate may have a significant influence on the rate of seed germination. Under the conditions of this study, seedling growth on saw-dust was very unsatisfactory compared to that was obtained from other substrata. Its use will not permit optimal expression of the inherent qualities of *Andrographis* seed lots leading to false conclusions. But it may be considered that filter paper with sterilised condition will be better than sand as the merits are already discussed and may be recommendable for the A*ndrographis* seed germination testing. The sand also allowed the best combinations of water and oxygen availability for the seeds. Seed germination can take place in any kind of substrate as long as it allows for a good aeration. Importance of good substrate is an important factor for seed germination that has already been justified.

Table 1: Comparison of means and variation for germination and other seedling traits.

Substrata	Germination index		Germination percent (%)		No. of secondary roots	No. of secondary roots	No. of primary leaves	No. of primary leaves
	Sterilized	Non-sterilized	Sterilized	Non-sterilized	Sterilized	Non-sterilized	Sterilized	Non-sterilized
Sand	22.057	46.54	79	78	8	4.45	2	2
Saw dust	9.452	16.785	36	60	1.95	4.45	2	2
Filter paper	32.25	31.267	69	72	12.1	9.15	2	2
Soil	25.772	43.847	62	75	2.9	4.75	2	2
SE	3.097	4.397	5.933	5.094	1.525	0.743	0	0
CV% (σ)	42.866	39.353	29.878	22.145	75.757	40.421	0	0

Any two means differ significantly from each other at P=0.05

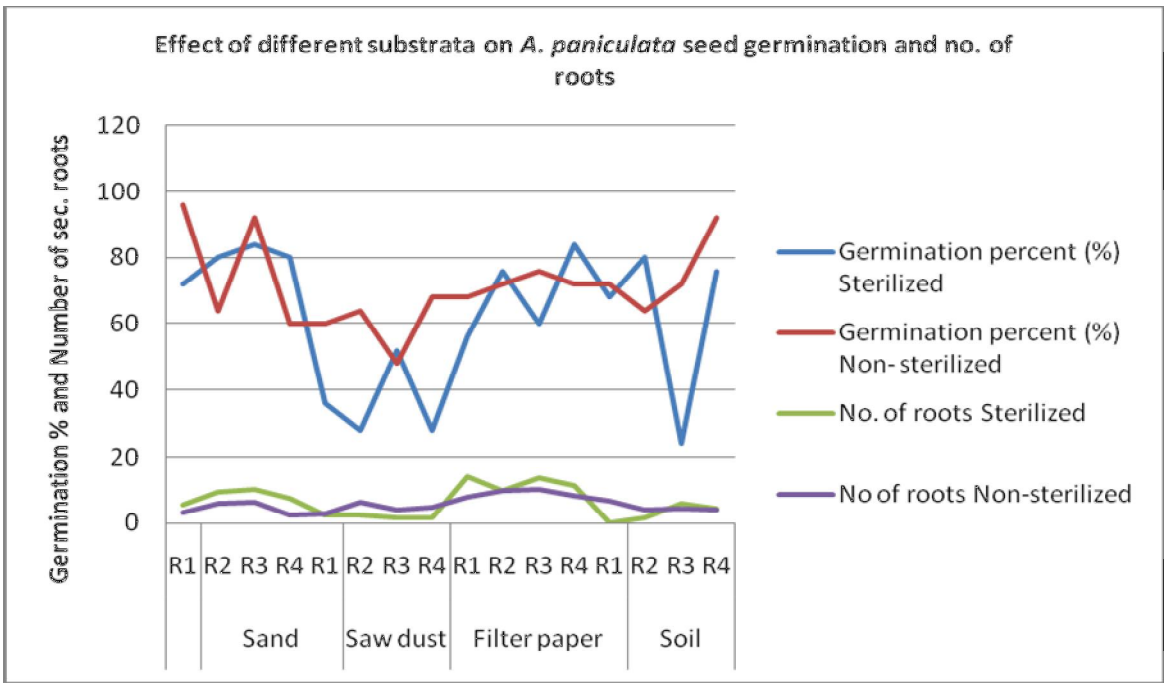

Fig. 1: Assessment of compatible substratum for *A. paniculata* seed germination testing.

Conclusion

From the above findings it may be summarized that the filter paper with sterilised condition can be recommended for the *andrographis* standard seed germination testing due to uniform and quick germination with proper secondary roots and primary leaves. If the filter paper is not available it can be replaced by sand substratum as an alternate as it showed more uniform germination with good secondary roots.

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