Effects of Different Surface Sterilizers on Seed Germination and Contamination of King of Bitters (Andrographis paniculata Nees.)

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Abstract: Andrographis paniculata is a medicinal plant belongs to the plant family Acanthaceae. The plant is commonly propagated by seed. High contamination and low germination are two prevalent problems occur during germination and embryo growth stages. Approaches to decrease the seed contamination level and increasing germination percentage of Andrographis paniculata seeds were studied using a combination of chemical treatments with different sterilizer at different concentrations and exposure times in a factorial experiment based on Randomized Complete Block Design (RCBD) with three replications. Analysis of variance indicated that the effect of treatments were significant (p ≤ 0.01) on seed germination and contamination percentage (OP & CP), as well as on mean germination time (MGT), while non significant effects were observed on Number of Days to First Germination (NDFG) and Average Germination Percentage (AGP). The maximum (12.5%) and the minimum contamination percentage (2.2%) were observed in 4 weeks after treating in control treatment and treatment with 10% NaOCl for 10 min respectively. The results showed that 10% NaOCl for 10 min treatments is an effective option for decreasing the seeds contamination percentage in this plant.

Key words: Andrographis paniculata • Seed sterilization • Seed germination and contamination

INTRODUCTION

Seed germination and embryo growth are the most important stages in the life cycle of plants in both agriculture and natural ecosystems. It often controls population dynamics, with major practical implications [1]. Using sterilizer agents such as sodium hypochlorite, ethanol, mercuric chloride, Tween 20, to produce high quality germinated seeds, are widely recommended [2]. In this regard, the efficiency of some of these chemical components used in the present study will be explained briefly. Hypochlorite is a very effective microbe killer that even in micro-scales is able to determinate microbial contaminants significantly. It is cheap and available that can be diluted to suitable concentrations. Ethanol is another strong sterilizing agent with extremely phytotoxic and for this reason the plant material should be exposed to it for a short period of time. According to [2], to improve effectiveness in sterilization procedure, ethanol is generally used prior to the treatment with other compounds like Tween 20. Mercuric chloride is extremely toxic to both plants and humans and must be disposed of with care. Since mercury is highly phytotoxic, it is important that many rinses be used to remove all traces of the mineral from the plant material.

Andrographis paniculata is one of the major medicinal plants commonly known as “The Great or King of Bitters” in the family of Acanthaceae [3]. It is an ancient medicinal herb with extensive ethno-botanical uses that grows abundantly in southeastern Asia, in moist and sunny situations [4, 5]. It is an erect annual herb with dark green and quadrangular stem, small leaves, white flowers, linear-oblong capsules and tiny yellowish brown seeds [6]. It is the source of several diterpenoids of which Andrographolide is the main compound with immunostimulant, antipyretic, anti-inflammatory and anti-diarrhea properties [4, 5]. The plant’s curative effects and economic aspects have been mentioned in related review articles [7].

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Propagation of *A. paniculata* generally occurs through seeds, although it has many germination problems. Saraswathy [8], in a study on the seed ecological aspects of *A. paniculata* reported problems in the seed germination. They assumed that seeds of *A. paniculata* possessed the combined dormancy of physical and innate nature. The seed germination in *A. paniculata* is infrequent and it is known to have low and irregular seed germination. On the other hand, the period of seed germination in *A. paniculata* is long and this leads towards increasing the contamination of the seeds and creating weak seedlings.

In order to reduce the aforementioned problem, this study utilized different sterilizer agents. Thus, the current study has attempted to explore the most appropriate method for sterilizing the seeds of *A. paniculata*.

**MATERIALS AND METHODS**

**Plant material and Chemicals:** Seeds of four different Malaysian accessions of *A. paniculata* including 11323, 11340, 11347 and 11349 were obtained from the Agro Gene Bank, University Putra Malaysia. The analytical grade chemicals used for the treatments, were supplied mostly from Sigma (Tween 20 and mercuric chloride sodium) and Fisher chemicals (Ethanol). The locally produced bleach solution containing 5% sodium hypochlorite was used instead of the expensive Sigma- Aldrich sodium hypochlorite solution.

**Experimental Technique:** The experiment was conducted in a controlled environment in Agro Gene Bank laboratory (Seed bank), located in Department of Crop Science, University Putra Malaysia between July and September 2010. Initial germination test indicated that germination periods were irregularly extended and contamination percentage was considerable in the employed accessions' seeds accordingly. Therefore, the decision was taken to test different sterilizer agents on seed germination and contamination of *A. paniculata*. For this purpose, an experiment was carried out with a Factorial based on Randomized Complete Block Design (RCBD) with two factors and three replications. The factors were consisted of *A. paniculata* accessions with four levels viz. 11323, 11340, 11347 and 11349 and different treatments (sterilizers) with six levels composed of 100% distilled water (control), 10% NaOCl for 10 min, 5% NaOCl for 10 min, 70% Ethanol + 100 μl of Tween 20 for 10 min, 0.2% mercuric chloride sodium for 5 min and 0.5% mercuric chloride sodium for 5 min. Consequently, a total of 24 treatment combinations were analyzed. Fifty seeds from each accession were soaked in separate Petri dishes in each level of treatments. Seeds were placed on Whatman No.2 filter paper moistened with 10 ml of distilled water in sterilized Petri dishes with 15 cm diameter. All dishes were sealed with a trip of parafilm to reduce water loss. During the study, average temperature was 32°C/26°C (maximum/minimum) and relative humidity (RH) varied between 60% and 75%. The germination percentage and contamination rate were recorded daily for 28 days. Mean Germination Time (MGT) was calculated to assess the rate of germination described by Ellis and Roberts [9].

\[
\text{MGT} = \sum_{0}^{n} \frac{N_i}{n}
\]

Where, \(N\) is the number of germinated seeds in the certain counting day, \(n\) is the total number of grown seeds and \(D\) is the number of certain day in each counting. The germination and contamination percentage (GP & CP) were recorded at the end of this period. The germination rate (GR) for each accession was calculated by dividing the germination percentage obtained at each counting to the certain number of counting day. The values gained from each count were then summed together to obtain the germination rate as below [10, 11].

\[
\text{GR} = \frac{\text{No. of germinated seeds}}{\text{Days to first count}} + \ldots + \frac{\text{No. of germinated seeds}}{\text{Days to final count}}
\]

Average germination percentage was calculated as follow:

\[
\text{AGP} = \frac{D_i}{\Sigma (1 / G_i)}
\]

Where, \(G_i\) is the percentage of seeds germinated for a certain day and \(D_i\) is the number of days after seed treatment (1 ≤ \(D\) ≤ 30).

**Statistical Analysis:** Initially, normality test of raw data was done using SPSS software version 16 and the square transformation method was employed for data transformation. The ANOVA analysis was performed and means comparison analysis was achieved using Duncan's multiple range test (\(P < 0.01\)).

**RESULTS**

Analysis of variance indicated that the effect of treatments were significant (\(p < 0.01\)) on germination rate and germination and contamination percentage (GR, GP and CP), as well as on mean germination time (MGT).
while non significant effects were observed on number of days to first germination (NDFG) and average germination percentage (AGP). The results of ANOVA revealed that the effect of accessions were significant (p<0.01) on properties of germination rate and germination and contamination percentage (GR, GP & CP), mean germination time (MGT) and also average germination percentage (AGP) but it was non-significant on number of days to first germination (NDFG). As it is described in Table 1, the interaction between treatment and accessions effects (T × A) was significant (p ≤ 0.01) for GP, MGT and CP traits. Consequently, it was revealed that interaction effects (T × A), are more influenced by treatment effect in this study (Table 1).

The maximum germination percentage was recorded for 5% Clorox (43.9%) and the minimum for 70% ethanol (26.7%) (Figure 1a). Significant differences were observed between the mentioned treatments as it has been presented in Table 2.

Among the subjected treatments, the highest contamination percentage belonged to control treatment (12.5%) in the fourth week of the experiment meanwhile, the lowest pollution was owned by 10% Clorox for 10 min (2.2%) (Figure 1b).

Germination and contamination percentage in accession 11323 both were higher than other accessions (Figures 2a & b). The treatment 70% ethanol evolved the lowest AGP in all studied accessions and 10% Clorox for 10 min caused the lowest contamination percentage (Figure 3a & b).

The correlation between germination percentage (GP) and mean germination time, average germination percentage and germination rate (MGT, AGP & GR) were significant and positive. While a negative correlation was observed between MGT and AGP. No significant correlation was found between contamination percentage and other measured traits (Table 3).
DISCUSSION AND CONCLUSION

Based on the obtained results, it was unveiled that suitable treatment for reducing contamination percentage in four studied accessions of *A. paniculata* was 10% Clorox for 10 minutes. This result was in agreement with some other upshots reported by different researchers [2, 12, 13]. Although the effect of different treatments were not significant in number of days to first germination, but as it is shown in Table 2 different treatments increased number of days to first germination and decreased germination percentage. It might be explained by destructive effect of ethanol and mercuric on embryo. The result of the current study matched up well with the findings of a similar conducted study [14], where ethanol and mercuric compounds decreased germination percentage of barley seeds. Deleterious effects of ethanol and mercuric compounds depend on two properties: the concentration and the exposure time. However, there were no significant correlation between CP and other measured traits, but the present study ratified that increasing of GP was followed by an increase in CP and a decrease in MGT. Anyway, the lowest rate of CP was occurred when the seeds were treated by 10% Clorox for 10 minutes.
Consequently, usage of 10% Clorox for seed sterilization is recommendable for *Andrographis paniculata* due to its higher efficiency, availability and reasonable price in compare with other sterilizers which are very expensive.

REFERENCES