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Effect of H_2SO_4 on Seed Germination and Viability of *Canna indica* L. Ornamental Plant

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Abstract

Canna indica L. roots are used for medicinal purpose. A decoction of the root with fermented rice is used in the treatment of gonorrhea and amenorrhea. The seed of canna is extremely hard, and needs to be "scarified" before sowing. The aim of the present investigation is to determine the hardiness problem of the seed. The seed sample was collected from the IARI, New Delhi in 2008. The work consists of Physical purity, standard germination test, seed vigour test. Experimental results has shown that, seed sample recorded the purity of seed (97.55 %) and seed sample showed the maximum germination percentage 95% after three and four hrs, H_2SO_4 scarification. The maximum root length (34.07 mm), maximum shoot length (23.62 mm) and maximum seedling shoot and root fresh weight (0.23 and 0.24 g) were observed at three, two hrs and control. H_2SO_4 scarification. The results indicated that H_2SO_4 scarification increase the germination percentage but it reduce the viability of the seed.

Key words: Canna, fresh weight, Germination, Scarification.

Introduction

Canna indica belongs to family cannaceae. *Canna indica* is a native of tropical America and is a very popular ornamental and Medicinal plant throughout the tropical world. *Canna indica* is an upright perennial rhizomatous herb. It having round, shiny black seeds**.** The seed of cannas is extremely hard, and needs to be "scarified" before sowing. Scarifying the seed can speed germination, especially if the seed has not swollen after being soaked. The seed are scarified generally with H_2SO_4. The seed usually germinates in 12days to 3 weeks. The plant is used in the treatment of women's complaints. A decoction of the root with fermented rice is used in the treatment of gonorrhea and amenorrhea. The plant is also considered to be demulcent, diaphoretic and diuretic. Seeds immersed in concentrated sulfuric acid (acid scarification) have been used to break seed coat dormancy in species of *Cercis*. According to Gebre and Karam (2004, scarification with concentrated (1.84 g cm-3 specific gravity) sulfuric acid for 15 min proved to be quite satisfactory in breaking the hard seed coat in *Cercis siliquastrum*. Jones and Geneve (1995) and Geneve (1991) considered scarification with concentrated sulfuric acid for 30 min for seeds of *C. canadensis* ample in order to allow for water imbibition. For breaking physical dormancy in many species, seed lots are sometimes scarified by chemical treatments such as sulfuric acid (Rolston 1978;

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Bilsland et al. 1984; Mahmoodzadeh et al. 2002; Ortega Baes et al. 2002; Elahifard et al. 2005; Ghadiri and Niazi 2005; Fang et al. 2006).

The objective of this paper investigation was to determine the hard seed problem in canna with sulphuric acid scarification effect on germination.

Materials and Methods

In order to study the effects of sulphuric acid scarification on germination and early seedling growth in canna, an experiment was conducted using a completely randomized design (CRD) with four replications. In this experiment, evaluated in five levels time of sulphuric acid scarification (0, 1, 2, 3 and 4 hour) by using H_2SO_4. This experiment was carried out at horticulture Laboratory, Department of Agriculture, University Azad of Jiroft Branch, Iran.

The seeds were sterilized by soaking in a 5% solution of hypochlorite sodium for 5 min. After the treatment, the seeds were washed several times with distilled water. 30 seeds were put in each petridish (with 9 cm diameter) on filter paper moistened with respective treatment in four replications. The petridishes were covered to prevent the loss of moisture by evaporation. The petridishes were put into an incubator for 14 days at 25 centigrade degrees temperature and 65% relative humidity. Every 24 hours after soaking, germination percentage and other traits were recorded daily. After 14 days of incubation, shoot and root length, shoot and root fresh weight, germination percentage and rate was measured. Seeds were considered germinated when the emergent root reached 2 mm length. Rate of germination, germination percentage and mean germination time were calculated using the following formulas (Mostafavi, 2011):

GP = SNG/SNO × 100%

Where: GC is germination percentage, SNG is the number of germinated seeds, and SNO is the number of experimental seeds with viability (Close and Wilson, 2002; Danthu et al., 2003).

GR=$\Sigma N/\Sigma(n\times g)$

Where: GR: Germination race; n: number of germinated seed on gth day and g: Number of total germinated

Seed Vigor = [seedling length (cm) × germination percentage]

Analysis of variance was performed using standard techniques and differences between the means were compared through Duncan's multiple Significant Difference test ($P < 0.05$) using SAS release 9.1 (SAS, 2002) software package.

Result and Discussion

Germination percentage

Results of scarification using sulfuric acid showed that increased time of sulfuric acid treatment caused germination percentage to growth; the highest germination percentage (90%) was observed in 4 hours treatment with sulfuric acid and the lowest percentage (10%) was obtained in treatment with distillated

water; these values are significantly different from each other. germination percentage variation during the experiment showed a fast increase of germination percentage from beginning of experiment until the 9^{th} day, after which was reduced or inhibited. Three or four hour treatments with sulfuric acid exhibited higher germination percentage compared to other treatments and the lowest germination percentage was observed in control group (tab 1).

Positive effect of sulfuric acid on seed germination observed in the present study has been also reported by many investigators (Fang et el., 1998; Herron and Clemens, 2001; Babashpour and Sharifivash, 2010; Chandra Joshi et el., 2010; Fordham, 1965; Gizachew, L and Scarisbrick, 1999; Noorafkan and Khoshkhui, 2005).

Investigating the germination enhancement and seed dormancy breakage in three species of hawthorn, the authors observed that scarification by sulfuric acid was more effective than other treatments (Mirzadeh vaghefi et al., 2009). As observed in our study, investigation in *Colutea sp* indicated that impermeability and hardness of seed coat is the major inhibitor of germination. Results of experiment conducted by (Olmez et al., 2009) on germination of *Armena Colutea* showed that sulfuric acid treatment had the highest effect on seed germination percentage.

The highest germination rate and percentage has been observed in treatment with sulfuric acid an investigation carried out on *Canna indica* L. the highest germination rate was observed when scarification was continued for four hours which occurred in the fifth day, the result was in agreement with those reported by (Olmez et al., 2009). Therefore it can be concluded that scarifying seed coat by sulfuric acid is a suitable method to overcome seed coat dormancy. Moreover, accelerated germination in seeds treated by sulfuric acid indicates that embryos of canna seeds are active and not dormant but the seed coat inhibits the embryo growth. Therefore, seed germination rate and percentage can be improved by removing seed coat using sulfuric acid.

Germination rate

Scarification by sulfuric acid indicated that increase in treatment time caused reduction of germination rate so that the highest germination rate was observed for 4-hour treatment in the fifth day and for 3-hour treatment in days 7, 9, 13, 11 and 15 (tab 2).

The lowest germination rate was observed in control group (distillated water) which showed significant difference to other treatments during experiment period. Table 2 shows that in the fifth day, germination rate was enhanced as a result of increased treatment time. According to (Herron and Clemens, 2001) scarification of *Melicytus ramiflourus* seeds by sulfuric acid, increasing treatment time from 15s to 30s and 60s causes increase of germination rate. Nasiri and isavand (2001) investigated effects of sulfuric acid on dormancy break and germination of asrasyd khsb night and carob seeds and reported that germination rate of asrasyd khsb night seeds was enhanced by increase of sulfuric acid level.

Plumule length, radicle length and their ratio

Scarification by sulfuric acid indicated that increase in treatment time enhanced radicle length. Results concerning radicle length showed that it was enhanced by increase in scarification time (four hours) (Noorafkan and Khoshkhui, 2005). Although there was no significant difference between fifth and seventh days, the treatment was significantly different from control. The highest variation of radicle

length for 3- and 4-hour treatment in days 11, 13 and 15 but the difference was not significant (tab 3 and 4).

Results of plumule length calculated by average length showed that by increase in scarification time in the 13th day the highest plumule length was obtained in 4-hour treatment and in the 15th day in 2-hour treatment (Chandra Joshi et al., 2010). Regarding the effect of scarification on radicle to plumule length ratio, it was observed that 4-hour treatment had the highest ratio of radicle to plumule length but the difference among various treatments was not significant (tab 2). In an experiment conducted to evaluate the effect of hot water and sulfuric acid on germination of tamarind and acacia, it was observed that the highest length of radicle and plumule was achieved when the tamarind seeds were treated with sulfuric acid 98% for 30 minutes (Khaleghe et al., 2009).

Fresh weight of root, shoot and ration of them

Results presented in the table showed that shoot fresh weight was increased by reducing acid concentration. The highest ratio of shoot to root fresh weight was obtained when the seeds were treated by sulfuric acid for one hour (tab 5).

Thus, from the discussion it may be concluded that the seed lot of *canna indica* L. showed good response in three hrs. H_2SO_4 treatment followed by two hrs. The results indicated that H_2SO_4 scarification increase the germination percentage but it reduce the viability of the seed.

Water impermeability of the testa is a physical exogenous dormancy according to Nikolaeva (1969). Concentrated sulphuric acid has been used for many years for softening of hard seed coats. (Hopkins, 1923).

CONCLUSION

It can be concluded that the best method for breaking dormancy of *Canna indica* L. which resulted in an increased germination percentage to 95% and gave highly quality of golden shower seedlings is acid scarification for 4 hour.

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Table 1- Mean comparison of different hour of H_2SO_4 scarification on Germination Percentage of canna

H_2SO_4	germination percentage (day)					
	5	7	9	11	13	15
One hr h_2so_4	42.5b	54c	60b	64b	66b	66b
two hr h_2so_4	83a	85b	88a	88a	89a	89a
three hr h_2so_4	90a	93a	94a	95a	95a	95a
four hr h_2so_4	90a	90b	94a	94a	94a	95a
control	9c	9d	9c	10c	10c	10c

Table 2- Mean comparison of different hour of H_2SO_4 scarification on Germination rate of canna

H_2SO_4	germination rate (day)					
	5	7	9	11	13	15
One hr h_2so_4	3.9a	1.92c	1.66b	1.45b	1.26b	1.09b
two hr h_2so_4	4.15a	3.03b	2.46a	1.99a	1.68a	1.48a
three hr h_2so_4	4.5a	3.31a	2.6a	2.15a	1.82a	1.58a
four hr h_2so_4	4.57a	3.24a	2.6a	2.13a	1.8a	1.58a
control	0.45b	0.31d	0.24c	0.22c	0.18c	0.16c

Table 3- Mean comparison of different hour of H_2SO_4 scarification on Root length of canna

H_2SO_4	root length (cm)				
	5	7	9	11	13
One hr h_2so_4	4.83ab	15.28ab	19a	23.54a	29.71a
two hr h_2so_4	7.03a	17.43a	19.19a	24.49a	31.23a
three hr h_2so_4	6.18b	13.75ab	18.47a	27.26a	34.07a
four hr h_2so_4	8.96a	17.46a	20.36a	27.06a	30.44a
control	1.21b	8.88b	14.4a	21.11a	21.78a

Table 4- Mean comparison of different hour of H_2SO_4 scarification on shoot length and ration of them of canna

H_2SO_4	shoot length (cm)		root length / shoot length	
	10	14	13	15
One hr h_2so_4	3.17b	22.75a	0.066ab	0.032b
two hr h_2so_4	13.51a	23.62a	0.15a	0.083a
three hr h_2so_4	2.03b	21.44a	0.02a	0.026b
four hr h_2so_4	0b	22.37a	0b	0b
control	0b	12.74a	0b	0b

Table 5- Mean comparison of different hour of H_2SO_4 scarification on Fresh weight root and shoot and ration of them of canna

H_2SO_4	fresh weight (g)		fresh weight root / shoot
	root	shoot	
One hr h_2so_4	0.24	0.23	1.23
two hr h_2so_4	0.2	0.21	1.08
three hr h_2so_4	0.22	0.19	0.91
four hr h_2so_4	0.21	0.17	1.13
control	0.16	0.12	1.22