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Effects of Seed Treatment on Removal of Physical Dormancy in *Canna indica* L.

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Authors' contributions

This work was carried out in collaboration between both authors. Authors KO and CAA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author KO managed the analyses and literature searches of the study. Both authors read and approved the final manuscript.

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ABSTRACT

The effects of different seed treatments on the germination of *Canna indica* L. were investigated. The seeds were subjected to chemical (sodium nitrate, sulphuric acid and hydrochloric acid), physical (hot water) and mechanical (puncturing the seed coat) scarification and allowed to germinate given a period of 16 days. The seeds were treated as follows: immersion in 50%, 70% and 100% sulphuric acid (H_2SO_4) and hydrochloric acid (HCl) for 20, 40, 60, 80, 100 and 120 minutes respectively, 24 hours soaking in 10 mM, 50 mM, 100 mM, 1000 mM Sodium nitrate ($NaNO_3$) and soaking in hot distilled water (70℃ and 100℃) for 5 and 10 minutes respectively. Mechanical scarification, chemical scarification using 70% HCl and concentrated sulphuric acid and hot water treatment (70℃) had considerable effect in promoting germination with maximum germination percentage of 50%, 35%, 88%, and 36% respectively. *Canna indica* seeds treated with $NaNO_3$ had the least germination (28%). However, treatment with concentrated sulphuric acid was most efficient in promoting germination (88%). Statistical analysis carried out indicated that there was significant difference at P=0.05 in the germination of seeds treated with hot water, mechanical scarification, sulphuric acid and hydrochloric acid at 70% and 100% but there was no significant difference in the germination of seeds treated with 50% of both hydrochloric acid and sulphuric acid respectively.

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Keywords: Dormancy; Canna indica; scarification; seed treatment; germination.

1. INTRODUCTION

Canna indica L. commonly known as Indian shot or Canna lily belongs to the family Cannaceae and is a wild cultivar found in the tropical regions [1]. According to Kessler, it can be seen in dump sites, abandoned gardens and moist places [2]. It is an invasive plant and even regarded as a weed [3]. *Canna indica* grows to a height of 2.5 m or more and it is a mesophyte colonial terrestrial plant [2]. It has underground rhizomes which are sympodial with chestnut cataphylls and abundant adaxial and abaxial roots. It possesses aerial shoots and they are 1-3 cm in diameter, with 7-11 sheating leaves. *C. indica* also has distichuous to spirally phyllotaxis, with light green foliar sheaths. The green leaves are glabrous with narrow ovate contours. The apices are twisted, acute and acuminate with cuneate decurrent asymmetric bases. The inflorescence is 40-41 cm long and intermediate, and each one bears 1-4 nodes. Each node has 2 flowers which are red. There are 6-8 nodes in the principal florescence and the basal internode has a length of 16-17 cm. The ovarium of *C. indica* is green and inferior and it contains spherical or sub-spherical pollen grains. The capsules are dark when mature, with 20-28 seeds that are ovoid in shape, black in color, with a smooth texture and diameter of 0.5-0.7 cm [1,2,3].

Abolaji et al. reported that the nutrients and biochemical in plants play an important role in sustaining humans and providing energy needed for life processes [4]. Ornamental plants have created a huge market for the grower. Such functional use of plants led to the development of ornamental plants market, which finally has reached a point which is economically significant. In 145 countries around the world, the cultivation of ornamental plants is carried out on a total area of 220,000 ha, and the trade volume of ornamental plants is around $50 billion USD [5,6]. Such advancement of the ornamental plants market made the researchers to be interested in various issues such as defining the cultivation methods, fertilization, pruning etc. and the studies are still going on [7,8]. Despite the economic importance of *C. indica* the seeds have a low degree of germination due to the possession of a hard seed coat [9]. This hard seed coat enables the seeds of *Canna indica* to survive long dormant periods [9]. Due to this hardness in the seed coat, the seed find it difficult to imbibe water, so it does not swell, thus remaining hard. Therefore, no germination is attainable under natural conditions of temperature and humidity [3]. According to Shaban, the percentage germination among different plant species varies and most seeds have critical moisture content at which germination occurs [10]. Germination percentage may also vary in seeds of the same plant; this may be due to fruits developing at different times and positions, or as seen in cucumber as a result of different seed positions in the same fruit [11]. Ali et al. and Babalola et al. in their separate works on members of Fabaceae family discovered that most of the members of the family possess physical dormancy [12,13]. It is therefore of importance that this plant should be studied in order to find ways of solving this dormancy and also to determine the proximate composition of the various parts of *C. indica*.

2. MATERIALS AND METHODS

2.1 Seed Collection

Mature *C. indica* seeds were collected from the field in Ozuoba Rivers State in March 2016 and properly identified by the Curator at the University of Port Harcourt Herbarium. The seeds used were fully matured and dried. Floatation method was used to determine the viability of the seeds.

2.2 Determination of Imbibition Rate

This was done using the procedure noted by Mensah and Ekeke [14]: The intact seeds of *C. indica* were cleaned and weighed. Thereafter, they were immersed in distilled water in petri dishes for 1, 4, 8 and 24 hours respectively. After the seeds had stayed in the water for the desired time, they were removed, cleaned and reweighed. The rate of imbibition was calculated using the procedures noted by Baskin and Baskin [15]. These are untreated seeds.

2.3 Mechanical Scarification

Mechanical scarification was done following the methods stated by Gunes et al. [16]. A small cut of 2 mm was done on the individual seed coat exposing the endosperm before placing in petri dishes. The seeds were placed in light conditions at a temperature of 25℃ and the numbers of germinated seeds were recorded for 16 days.

2.4 Hot Water Treatment

Seeds were immersed in hot water at 70℃ and 100℃ for 5 and 10 minutes respectively. They were then rinsed several times for two minutes under running tap with cold distilled water and place in petri dishes. The seeds were placed in light conditions at a temperature of 25℃ and the numbers of germinated seeds were recorded for 16 days.

2.5 Scarification with Sodium Nitrate

Seeds were soaked in 10 mM, 50 mM, 100 mM and 1000 mM of $NaNO_3$ respectively for 24 hours at room temperature. They were rinsed several times for two minutes under running tap with distilled water and placed in petri dishes. The seeds were placed in light conditions at a temperature of 25℃ and the numbers of seeds that showed radical protrusion were recorded for 16 days.

2.6 Scarification with Hydrochloric Acid and Sulphuric Acid

Intact viable seeds were soaked in 50%, 70% and 100% hydrochloric acid and sulphuric acid respectively. For each concentration, seeds were soaked at different time intervals 20, 40, 60, 80, 100 and 120 minutes, after which they were rinsed several times with distilled water and placed in petri dishes. The seeds were placed in light conditions at a temperature of 25℃ and the numbers of seeds that showed radical protrusion were recorded for 16 days.

2.7 Statistical Analysis

Data obtained from the experiment were subjected to statistical analysis using Microsoft Excel (2010). Comparisons of mean germination for each treatment were done using analysis of variance (ANOVA). The least significant difference test was used to separate the means at *P =0.05*.

3. RESULTS AND DISCUSSION

3.1 Imbibition Rate

Intact seeds of *C. indica* without any pretreatment (control) showed very poor germination of 7.5% after 16 days. The seeds of *C. indica* showed a very low rate of imbibition. The rate of water uptake increased progressively from 0.29% after 1 hour immersion to 9.20% after 24 hours immersion (Fig. 1).

3.2 Mechanical Scarification

Mechanical scarification of *C. indica* seeds increased the germination percentage considerably (50%) compared to the control (7.5%) after 16 days (Fig. 2). There was significant difference at *P=0.05* between the intact seeds and the mechanically scarified seeds (Table 1).

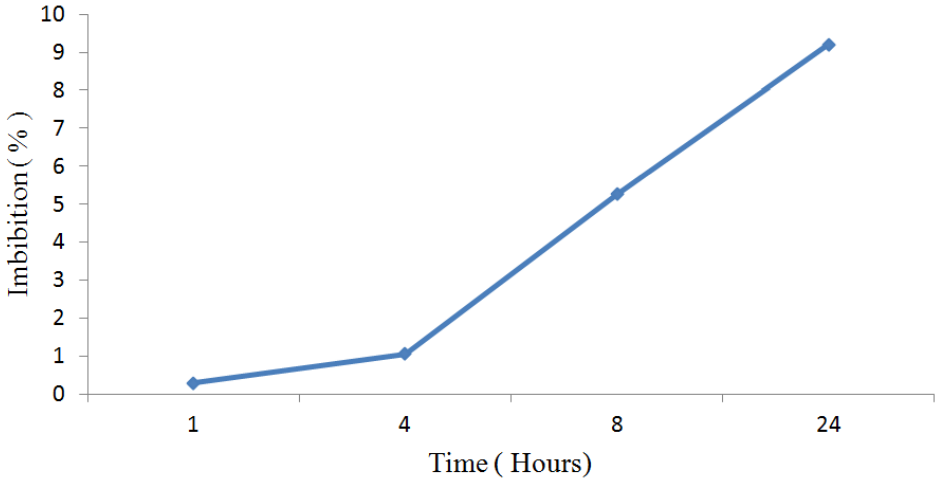

Fig. 1. Percentage imbibition of *C. indica*

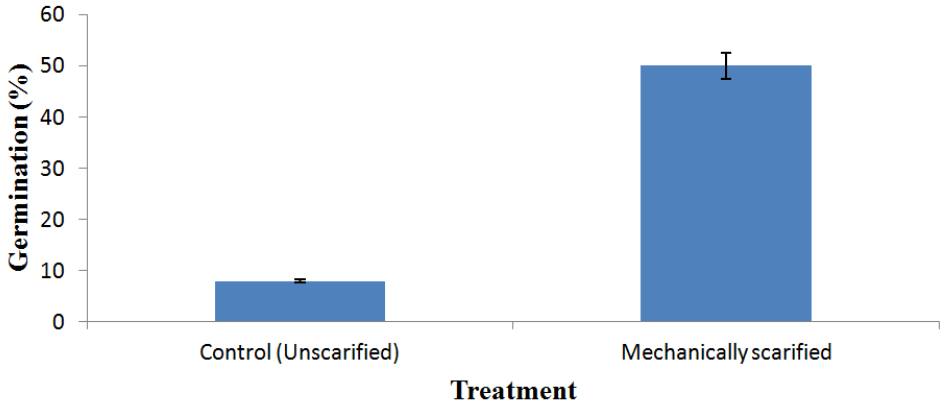

Fig. 2. Germination rate of *C. indica* seed under different treatments

Table 1. Mean germination percentage of intact seeds and mechanically scarified seeds after 16 days

Treatment	Mean germination percentage
Control	1.50[b]
Mechanical scarification	10.00[a]
LSD	4.41

Values with the same alphabet are not significantly different at 5%

3.3 Hot Water Treatment

Generally, all hot water treatments enhanced the percentage germination of the seeds compared to the control treatment. There was significant difference at *P=0.05* among the various treatments (Table 2). The results showed that seed germination increased with increased soaking period and maximum number of geminated seeds (41%) was found in imbibition in hot water at 70℃ for 10 minutes. Increasing the temperature of the hot water to 100℃ caused a decrease in the number of germinated seeds. The least number of germinated seeds was observed in seeds immersed in hot water at 100℃ for 10 minutes after 16 days (Fig. 3).

Table 2. Mean germination time of seeds treated with hot water

Treatment	70°C	100°C
Control	1.50[b]	1.50[b]
5 minutes	7.25[a]	4.75[a]
10 minutes	8.25[a]	2.50[a]
LSD	2.64	1.38

Note: For each treatment, values with the same alphabet are not significantly different at 5%

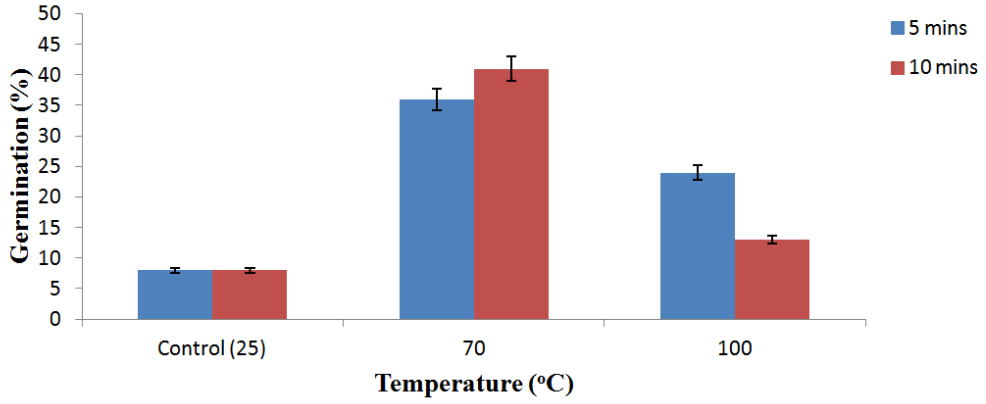

Fig. 3. Germination rate of *C. indica* seeds after hot water treatment

3.4 Treatment with Sodium Nitrate

C. indica seeds soaked in $NaNO_3$ for 24 hours had little effect on seed germination as compared to other treatments. Total germination (28%) was seen in seeds soaked in 10 mM and 1000 mM $NaNO_3$ respectively after 16 days (Fig. 4). There was significant difference between the treatments at *P=0.05* (Table 3).

3.5 Scarification with Hydrochloric Acid

Scarification of *C. indica* seeds with HCl induced germination in the different concentrations. In 50% HCl, maximum germination (35%) was seen at 80 minutes while in 70% HCl, maximum germination was seen at 60 minutes after 16 days. In both concentrations, increasing the soaking time caused a decline in percentage germination. More so, increasing the concentration of HCl to 100% also caused a decrease in germination as compared to 50% and 70% HCl (Fig. 5). There was no significant difference between treatments in seeds treated with 50% HCl but there was significant difference in treatments with 70% and 100% HCl (Table 4).

Table 3. Mean germination of seeds treated with $NaNO_3$

Treatment (mM)	Mean germination
Control (0)	1.50^{c}
10	5.50^{a}
50	3.75^{b}
100	2.00_{c}
1000	5.50^{a}
LSD	1.64

Note: Values with the same alphabets are not significantly different at 5%

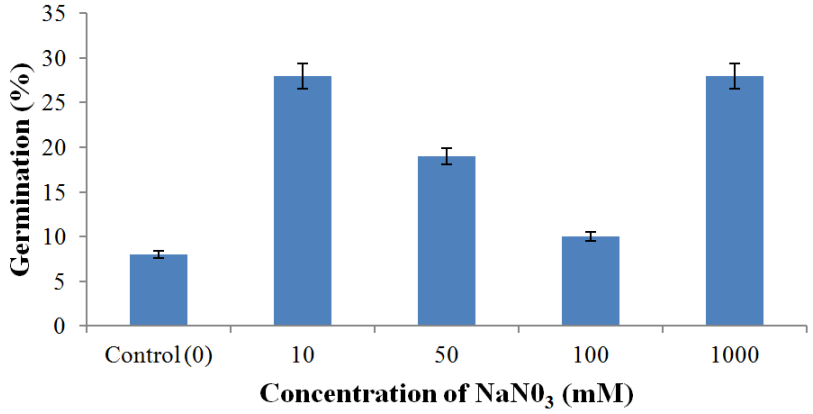

Fig. 4. Germination rate of *C. indica* seeds treated with $NaNO_3$

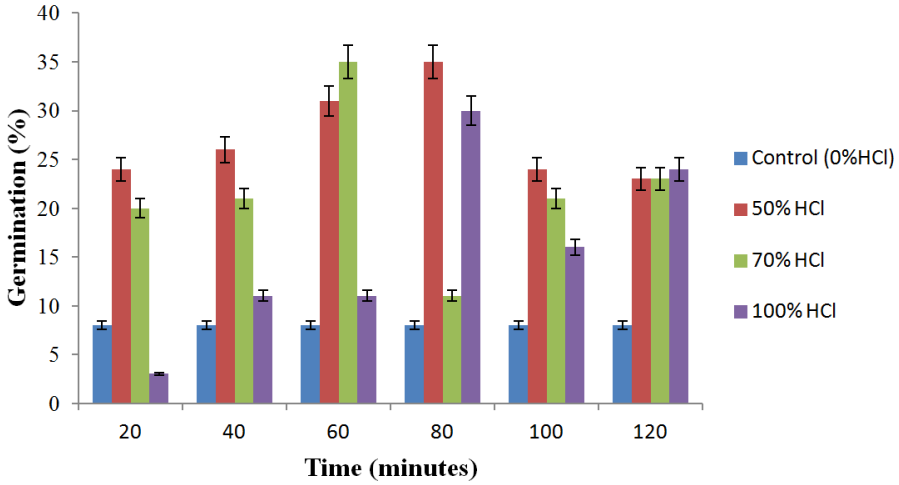

Fig. 5. Germination rate of *C. indica* seeds treated with HCl

3.6 Scarification Using Sulphuric Acid

Scarification of seeds with 50% H_2SO_4 had no significant effect. Increasing the concentration of H_2SO_4 to 70% showed a little increase in germination with maximum germination of 35% obtained at 100 minutes. Increasing the soaking time further to 120 minutes caused a decrease in germination (31%). Increasing the concentration of the acid to 100% had a great effect on the germination of the seeds as maximum germination of 88% was obtained at 100 minutes (Fig. 6). There was significant difference in seeds treated with 70% and 100% H_2SO_4 (Table 4).

3.7 DISCUSSION

C. indica seeds showed a very low imbibition rate. This shows that the seed has a hard seed coat which hinders the absorption of water. This causes poor germination in the seed since there is not enough water to trigger germination. Similar results were obtained by Mensah and Ekeke, while working on the seeds of *Senna obtusifolia,* they observed that water uptake in the intact seeds was very low (13.5%) after 24 hours exposure [14]. They concluded that the hard seed coat of the plant might retard the absorption of water. Graven et al. earlier reported that seeds of *C. indica* placed on a wet filter paper or in water at 40-59℃ temperature overnight opened their imbibition lid after 24 hours in the dark [9]. Maria also reported that since the seeds of *C. indica* cannot imbibe sufficient water due to their hard seed coat, germination will not occur [3]. Germination of intact seeds of *C. indica* without any pretreatment showed very poor germination (7.5%) although Graven et al. reported that *C. indica* seeds will show no germination without pretreatment, this difference in results could be due to the difference in the climatic environments of the crop [9]. The poor germination rate and percentage shows that the seed has a hard seed coat which needs some pretreatment to soften it, so that it can imbibe water for germination to occur. Germination can be affected, from chemicals that get into the seed, after seed soaking to soften the hard seed coat, from plant growth regulators [17,5,6] and stress factors [18,19]. It was found that dormancy breaking treatments significantly affected germination rate of *Sphaerophysa kotschyana* seeds [20].

Mechanical scarification of the seeds had significant effect and improved the germination considerably (50%) compared to the control. This is in line with the work of Babalola et al., they showed that mechanical scarification increased the germination of *Cassia fistula* from 12% to 41% [13]. Also, Sadeghi et al. reported that mechanical scarification increased the germination percentage (81%) of the seeds of *Rubia tinctorum* [21]. This shows that mechanical scarification is an excellent way of overcoming dormancy caused by hard seed coat.

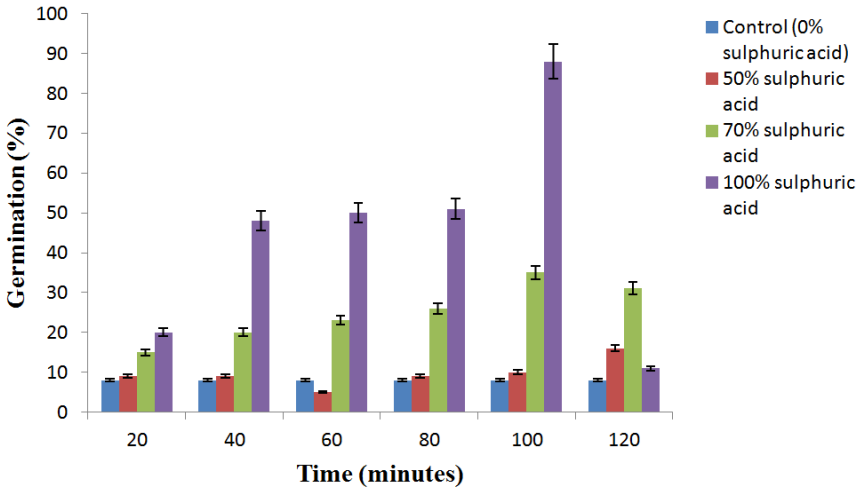

Fig. 6. Germination rate of *C. indica* seeds treated with H_2SO_4

Table 4. Mean germination of different concentrations of HCl and H_2SO_4

Duration (minutes)	HCl			H_2SO_4		
	50%	70%	100%	50%	70%	100%
Control (0)	1.50^{a}	1.50^{cd}	1.50^{c}	1.50^{a}	1.50^{d}	1.50^{c}
20	4.75^{a}	4.00^{bcd}	1.00^{c}	1.75^{a}	3.00^{cd}	4.00^{c}
40	5.25^{a}	4.25^{ad}	2.25^{c}	1.75^{a}	4.00^{bcd}	9.75^{b}
60	6.25^{a}	7.00^{a}	2.25^{bc}	1.00^{a}	4.50^{ad}	10.00^{b}
80	7.00^{a}	2.25^{cd}	6.00^{a}	1.75^{a}	5.25^{ac}	10.25^{b}
100	4.75^{a}	4.25^{ac}	3.25^{bc}	2.00^{a}	7.00^{a}	17.50^{a}
120	4.50^{a}	6.50^{ab}	4.75^{ab}	3.25^{a}	6.25^{ab}	2.25^{c}
LSD	0.00	2.77	2.56	0.00	3.04	3.08

Note: For each treatment, values with the same alphabet are not significantly different at 5%

C. indica seeds soaked in hot water also improved the germination considerably compared to the control. There was an increase in seed germination from 36% to 41% for seeds soaked in 70℃ for five and ten minutes respectively and also a decrease in germination from 24% to 13% at 100℃ for 5 minutes and 10 minutes respectively. This shows that germination decreases with prolonged exposure of the seeds to hot water at a very high temperature. This result supports the work of Sadeghi et al., they observed that seeds of *R. tinctorum* soaked in hot water at 90℃ for 5 minutes gave a maximum germination percentage of 78%, prolonging the soaking period caused a decrease in the germination of the seed [21]. This indicates that soaking seeds in hot water may rupture the seed coat allowing water to enter into the seed, thus triggering germination. However, prolonged exposure of the seeds to hot water may destroy the embryo.

Scarified seeds in $NaNO_3$ had little effect on the germination percentage of the seeds, a maximum germination of 28% was observed in seeds soaked in 10 mM and 1000 mM. This result agrees with the work of Ali et al., they observed that *Rynchosia capitata* seeds soaked in HNO_3 had little effect on the seed germination (17%) while seeds soaked in KNO3 had no effect on the seed germination [12]. This indicates that nitrates treatments are not efficient for breaking dormancy. Scarification of *C. indica* seeds with HCl had considerable effect on the germination of the seeds in the various treatments. Maximum germination was seen in 50% and 70% HCl at 80 minutes and 60 minutes respectively. Increasing the soaking time and the concentration of the acid reduced the germination of the seeds. Data provided by Ali et al. agrees with this result, they reported that soaked seeds of *R. capitata* in HCl (36%) increased the germination of the seed (90%) [12]. Increasing the concentration (70%) of the acid and the soaking time (18 hours) caused a reduction in the germination of the seeds (35%). This could be due to the fact that prolonged exposure of seeds to HCl in high concentration could cause damage to the embryo, thus destroying it instead of breaking dormancy. Acid scarification of *C. indica* seeds using H_2SO_4 for 100 minutes gave the best result (88%). Seeds soaked in 50% H_2SO_4 had little effect on the seed germination while the concentration to 70% increased the germination considerably. This is in line with work of Babalola et al. which revealed that *Cassia fistula* seeds soaked in diluted acid showed a very low germination percentage (3-9%) [13]. The increased soaking time and the concentration of acid from 100 minutes to 120 minutes caused a reduction in the germination of the seed (11%). This may be due to the fact that the acid damages the embryo on prolonged exposure. Similar results were obtained in experiments with *Rynchosia capitata* [12], *Rubia tintorum* [13], *Cassia fistula* [13] which showed that concentrated H_2SO_4 increased their germination considerably, and on prolonged exposure damaged the seed.

4. CONCLUSION

The study showed that the seeds of *C. indica* have physical dormancy due to the possession of a hard seed coat. Treating the seeds before sowing softened the seed coat thus enhancing germination. Treating seeds with hot water, mechanical scarification, HCl and H_2SO_4 showed a considerable effect on the germination of the seed compared to the control. H_2SO_4 had the highest effect on germination with a maximum percentage germination of 88% while $NaNO_3$ had the least effect on germination with a maximum

percentage germination of 27.5%. Concentrated H_2SO_4 can be recommended as a seed treatment for breaking dormancy due to a hard seed coat.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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