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South African Journal of Botany 83 (2012) 193 – 199

SOUTH AFRICAN JOURNAL OF BOTANY

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The effect of sulphuric acid and effective micro-organisms on the seed germination of *Harpagophytum procumbens* (devil's claw)

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Received 11 October 2011; received in revised form 14 May 2012; accepted 29 May 2012
Available online 11 October 2012

Abstract

The present study investigated the germination enhancement of *Harpagophytum procumbens* using sulphuric acid and effective microorganisms. *H. procumbens* is a geophyte that occurs mainly in central, east and south east of Namibia where it was previously regarded as a nuisance due to its fruit-claws getting caught on sheep and other livestock. *H. procumbens* seeds were pre-treated in effective micro-organisms (EM) resulted in a germination rate of 32%, while those pretreated with sulphuric acid H_2SO_4 germinated to 17% compared to 5.3% that germinated from the control. The combination of EM and H_2SO_4 resulted in a lower germination percentage than as expected. The study concludes that sulphuric acid and effective micro-organisms enhance germination in *H. procumbens*. It is therefore recommended that the two treatments be considered to *H. procumbens* stakeholders who have been struggling with the germination of the species. Follow up research is required.

Keywords: *Harpagophytum procumbens*; Effective microorganisms; Sulphuric acid; Germination; Seedlings; Dormancy

1. Introduction

Harpagophytum procumbens is a geophyte with a positively gravitropical tuberous main root, from which plagiotropical thick secondary roots develop. The genus *Harpagophytum* is composed of two species (*Harpagophytum zeyheri* and *H. procumbens*) which are perennial herbs with creeping stems that sprout every year from the main root. Secondary root tubers, which can reach a length of 5–25 cm, grow from the main root (parent tuber). It is these secondary tubers that are harvested for medicinal purposes containing active ingredients that have analgesic and anti-inflammatory properties (Cole and Strohbach, 2005). The plant is called devil's claw because of the very sharp and hooked form of the fruit (Cole and Strohbach, 2005). The fruit comprises a flattened woody capsule with spiny appendages on each carpel (Hachfeld, 2003). Recruitment rates are low with only a few seedlings surviving the first year. Despite these life history traits, *H. procumbens* is considered a pioneer or even 'weedy' species and is often found growing in areas where the soil has been disturbed or where grazing pressure is high. In established plants, annual shoot growth from the perennial tuber begins after summer rain (usually October/November) and the shoots die back between April and June as a prelude to winter dormancy (Cole and Strohbach, 2005).

Harpagophytum species occur between 15 degrees and 30 degrees latitude in the Southern hemisphere. *H. procumbens* in particular, is found in Namibia, Botswana and South Africa (Fig. 1). Indigenous people of Southern Africa have used *H. procumbens* medicinally for centuries, if not millennia. The tuber is traditionally used for fever relief, blood diseases, muscular aches and pains, and as an analgesic during pregnancy. In addition, pulverized root material is used as an ointment for sores, ulcers and boils, and for difficult births. Today there is a widespread use of the plant by indigenous inhabitants of Namibia and other stakeholders (Grote, 2003).

H. procumbens plants were first collected and described by European scientists in 1820 and the medicinal value was discovered in 1907 by G.H. Mehnert, in Namibia (Grote, 2003)

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doi:10.1016/j.sajb.2012.05.006

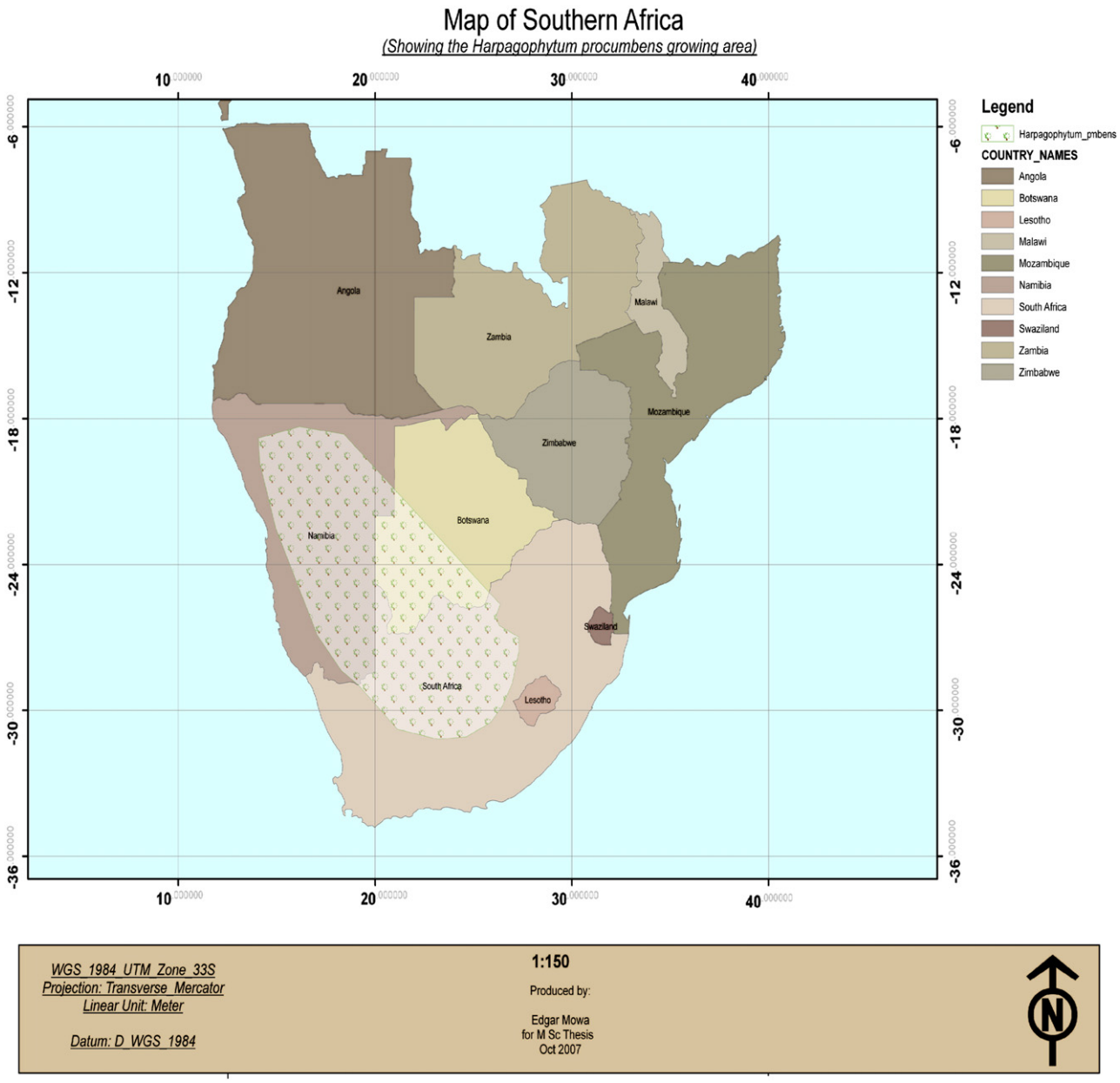


Fig. 1. Distribution of *H. procumbens* in Southern Africa.
H. procumbens zone on the map adapted from CITES (unpublished).

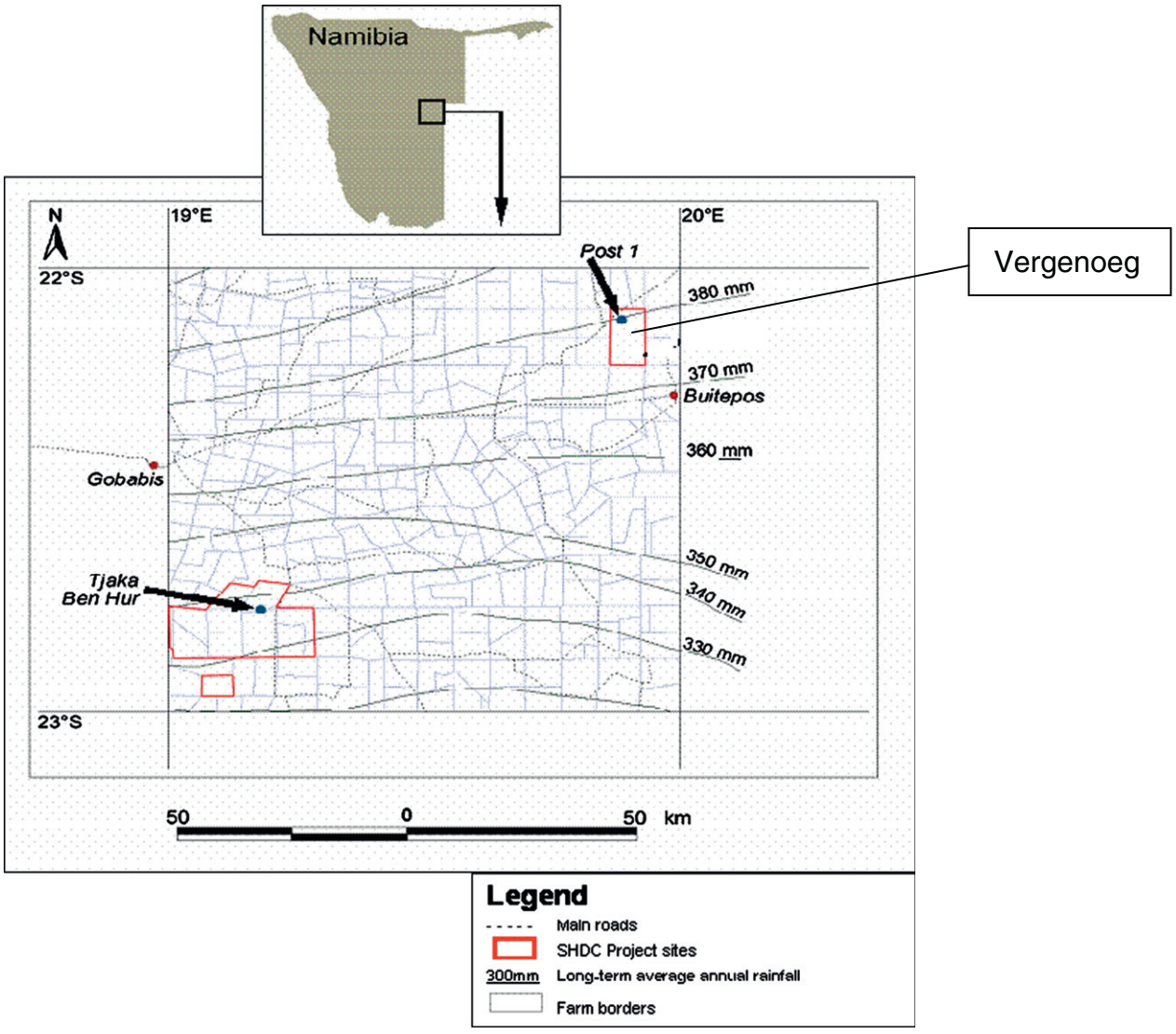
for 'western medicine'. Some dried *H. procumbens* tubers were then exported to Germany, where they were studied by B. Zorn at the University of Jena in 1950, and in 1962 the Namibian company Harpago (Pty) Ltd started exporting *H. procumbens* in larger quantities. This international demand resulted in additional pressure being exerted on the resource itself which in turn led to questions regarding its sustainability. The indigenous inhabitants of Namibia, Botswana and South Africa have increasingly come to depend on harvesting and selling the plant for their livelihoods (Grote, 2003). Namibia is the world's largest supplier of *H. procumbens* in the international market (Raimondo and Donaldson, 2002).

Of the total 6 269 297 kg exported from 1992 to 2006 (Table 1), 95% of exports originate from Namibia, 3% from Botswana, and 2% from South Africa (Cole and Bennett, 2007). The demand for *H. procumbens* in the international market is unpredictable. As the lead supplier, Namibia has the potential to affect the world market price for devil's claw (Cole and Bennett, 2007). Though demand may be unpredictable, consistent availability of supply has to be taken care of at all times, so that benefits continue sustainably.

The main importing countries of Namibian *H. procumbens* are Germany, France, Italy and Britain (Lombard, 2003). Germany leads among the four (4) above-mentioned countries, for example, one (1) German company (with branches in France, Spain, Italy and Poland) purchased 41%, 44% and 23% of Namibia's exports in 2002, 2003 and 2004 respectively. From 2003 to 2005, only nine companies (five German, two

French, one Spanish and one South African) purchased more than a container load of devil's claw in a single year (Cole and Bennett, 2007). In order to meet market demand and ensure sustainability, management measures have to be put in place so that the plant resource continues to provide these benefits over a prolonged period. Part of these management measures is through regeneration from *H. procumbens* seeds both under domestication and to some extent in the wild. This is needed because the supply of seedlings to *H. procumbens* farmers remains a constraint to its cultivation and therefore a reliable means of continuous supply seedlings is desired. The supply of seedlings is hampered by poor germination rates of *H. procumbens*. Recent studies in Namibia have shown that the current germination rate of *H. procumbens* in Namibia varies from 1–2% to 37% (Kelly, 2003; Kumba et al., s.a.) which are still considered to be low. The rationale for these poor germination rates has been understood to be the plant's testa known to influence dormancy. On the one hand, there have been several experiments to enhance germination rates in *H. procumbens* but their results still leave much to be desired. For instance, an experiment using sulphuric acid (H_2SO_4) resulted in no germination at all (Ernst et al., 1988). Considering that sulphuric acid has been widely used to enhance germination of several species (Samarah and Abu-Zanat, 2005), the results of that study with no germination at all become questionable.

Another successful germination enhancer of several species with dormant germination characteristics such as *H. procumbens* is the effective micro-organisms (EM). The concept of EM was developed by Higa and James (1994). EM consist of mixed cultures of beneficial and naturally-occurring micro-organisms that can be applied as inoculants to increase the microbial diversity of soils and plant. It is composed mainly of lactic acid bacteria, photosynthetic bacteria, yeasts, and actinomycetes that are commonly found in the soil. All of these are mutually compatible with one another and can coexist to increase



Map 1. Two sites for harvesting fruits: Vergenoeg and Tjaka and Ben Hur Communal Development Center. GIS files adapted from NARIS (AEZ, 2001).

microbial diversity (Higa and James, 1994). The microbes in EM have the ability to breakdown the organic matter thus releasing beneficial soluble substances such as amino acids, sugars, alcohol, hormones and similar organic compounds that are absorbed by plants (Higa, 1999).

Research has shown that the inoculation of EM cultures to the soil/plant ecosystem can improve soil quality, soil health, and the growth, yield, and quality of crops. Research has further found that EM increase seed germination and vigor in various species of carrot, cucumber, pea, beet, and tomato species. Khan et al. (2006) present further evidence that EM (1% and 2%) enhance seed germination. Though species investigated above do not possess hard seed coat, it would be interesting to know if the same principle applies to hard-coated seeds like those of *H. procumbens*. The understanding of germination enhancement through EM and H_2SO_4 is important to potential suppliers of seedlings for cultivation of *H. procumbens*. This will enhance the advancement in the sustainable management of the species, which got concerns from stakeholders who were anxious of the future of *H. procumbens*. This paper presents an investigation on breaking dormancy in *H. procumbens* seeds using different concentrations of sulphuric acid and effective microorganisms.

2. Materials and methods

2.1. Data collection

A total of six-hundred and sixty (660) seeds from fruits collected at Tjaka Ben-Hur, south-east of Gobabis and Vergenoeg east of Gobabis in Namibia were used for this experiment overall (Map 1). Fruits were collected randomly from several plants in the wild. Seeds from these fruits were dried and cleaned to avoid adverse effects like loss in viability and growth of fungus (Dickie et al., 1984).

2.2. Seed viability

Seed viability, among other factors, can influence a seed's ability to germinate (Bernal, 1953). Therefore, a seed viability test was done to estimate the percentage of viable seeds. The results from the test would help in deciding whether it was worthwhile to apply dormancy-breaking treatments considering the proportion of seeds that remain dormant despite using dormancy-breaking treatments (Zhang et al., 2006). The 2,3,5 triphenyltetrazolium chloride (TTC) test (Bernal, 1953) was in this regard used to test for seed viability.

From the initial six-hundred and sixty (660) seeds, one-hundred and fifty (150) seeds were used to test for seed viability with a representative sample (Salant and Dillman, 1994). *H. procumbens* seeds are 6–8.5 mm long and 3–5 mm wide in size (Ernst et al., 1988). The average weight of each seed is 13.90 ± 5.98 mg. When imbibed in water for 24 h, these seeds will increase a moisture content of 227.8 ± 59.9% of the seed dry weight. These seeds have a gray or black seed coat color. Under the endosperm lies a straight, white embryo. These seeds were imbibed in water for 24 h after which they were each bisected through the embryo with a razor blade. One half of the seed was discarded and the other was placed in 1% TTC (staining) solution for 1 h in darkness at 30 °C (because of the effect of ultra violet light on the solution's color). The stained seeds were then washed three times in distilled water to remove excess TTC in order to make the embryo transparent, making them easier for evaluation (Ellis et al., 1985). Seeds that showed to be stained (pink) on their embryos were recorded as viable and those that were not stained were recorded as non-viable. The TTC test was used this way because the methodology has been recommended for members of the Pedalicae family known to be hard seed-coated (Ellis et al., 1985).

2.3. Seed germination

The remaining five-hundred (500) seeds were then washed in 10% Jik solution to avoid any fungal developments (Awodele et al., 2007). A total of one hundred (100) seeds were used for each of the five (5) treatments which were replicated five (5) times of 20 seeds per replicate. These seeds were then used for the germination experiment as described below.

Five (5) different treatments for the germination experiment were set-up as follows:

a) pre-treatment of seeds with 18% sulphuric acid for 20 min (H_2SO_4 18%);
b) pre-treatment of seeds in 50% sulphuric acid for 20 min (H_2SO_4 50%);
c) pre-treatment of seeds in 1% effective micro-organisms for twenty-four (24) hours (EM 1%);
d) pre-treatment of seeds in 2% effective micro-organisms for twenty-four (24) hours (EM 2%); and
e) soaking seeds only in water for 24 h to serve as the experiment's control (Control).

Table 1
Unprocessed devil's claw exports, 1992–2006.
Table adopted from Cole and Bennett (2007).

Devil's claw exports (kg)				
Year	Botswana	South Africa	Namibia	Total
1992	10 719	No data	96 000	106 719
1993	3279	No data	66 000	69 278
1994	24 437	No data	158 000	182 437
1995	45 633	No data	284 409	330 042
1996	No data	No data	313 652	313 652
1997	5493	No data	251 091	256 584
1998	501	No data	613 336	613 837
1999	2050	6936	604 335	613 321
2000	No data	341	379 740	380 081
2001	33 506	31 112	726 333	790 951
2002	27 950	20 619	851 016	899 585
2003	3084	4500	592 387	599 971
2004	42 025	14 000	331 466	364 253
2005	540	27 000	336 713	361 095
2006	2249	No Data	358 846	361 095
TOTAL	201 465	104 508	5 963 324	6 269 297

After the above-mentioned treatments, sand with ferralic arenosols from Vergenoeg was prepared as a growth substrate. This sand was prepared in accordance with Justice (1972) and Hanson (1985) who recommended the growth substrate to be sterilized in order to be non-toxic. As indicated earlier, sand as a substrate is mostly used for large seeded species with longer germination periods and was therefore preferred as *H. procumbens* which has dormancy that presents it with longer germination periods (Ellis et al., 1985).

Seeds were then sown according to their treatments in 25 × 20 cm bags of sand at 2 cm depth (Ellis et al., 1985). They were placed in a growth chamber set at 10 °C for 12 h and 38 °C for 12 h with the higher temperature being in light. The daily alternating temperature was used in accordance with Kok (1986), who suggested that alternating very high and very low temperatures could be a trigger for *H. procumbens* germination.

Each bag was irrigated with 100 ml of water daily for 3 weeks considering the water-holding capacity of the bags of sand used. Light was set for fourteen (14) h and ten (10) h in darkness for the whole germination period. This was simulated considering that summer days are longer than nights, when *H. procumbens* germinate in the field. The response variable which in this experiment was the numbers of seedlings germinating per bag and subsequently per treatment was recorded. The recording was done for three (3) weeks on a daily basis from the sixth day after they were sown.

2.4. Data analysis

Data was tested for normality using the Kolmogorov–Smirnov normality test in SPSS. The one-way analysis of variance (ANOVA) using the SPSS statistical program was employed to test for mean differences in the number of seedlings germinated between treatments (Field, 2005). In order to determine the differences between the treatments, the least significant difference test (LSD) was used (Field, 2005).

3. Results and discussions

The Kolmogorov–Smirnov normality test indicated that the germination data of *H. procumbens* seeds were normally distributed ($p > 0.05$). Table 2 shows that eighty-two percent (82%) of seeds tested were viable while the remaining eighteen percent (18%) were non-viable. This may be attributed to the fact that some seed capsules were found empty. Furthermore, other seed capsules were partially damaged by some insect pests which infested containers where these seeds were kept for a period since being harvested from seed pods. Table 3 shows that pretreated seeds initialized germination in the first week compared to the untreated seeds from the control which showed no germination till the end of the first week. Most seedlings of pretreated seeds (except seeds pretreated in EM) germinated in the second week whereas most seedlings from the control germinated in the third week. This implies therefore that pretreatment of *H. procumbens* seeds with sulphuric acid and/or effective micro-organisms cuts down on the germination period. This could be beneficial to *H. procumbens* stakeholders who germinate the species to supply to farmers in cases where demand is high for seedlings.

Table 2
Seed viability test results.

Total fruits	Total seeds	Sample size	Percentage viability	
			Viable	Non-viable
10	660	150	82% ±	18% ±

Table 3
Total seedling germination per week per treatment.

Treatment	Total germination by week		
	Week 1	Week 2	Week 3
H_2SO_4 18%	9	14	12
H_2SO_4 50%	12	19	16
EM 1%	11	25	12
EM 2%	13	35	14
Untreated	0	2	7

Data indicated that there were significant differences between the germination per treatment ($p < 0.05$). This is because of different enhancing capabilities of each treatment. Fig. 2 shows that the pre-treatment of seeds with EM 2% gave the highest germination percentage followed by EM 1% compared to the lowest germination percentage with untreated seeds. This indicates that the concentrations of EM have a marked role in determining the germination rate of *H. procumbens*. Pre-treatment of seeds with 18% and 50% sulphuric acid gave 35% and 47% germination respectively compared to the control (9%) (Fig. 2). This is attributed to the fact that scarification of seeds with sulphuric acid (H_2SO_4)

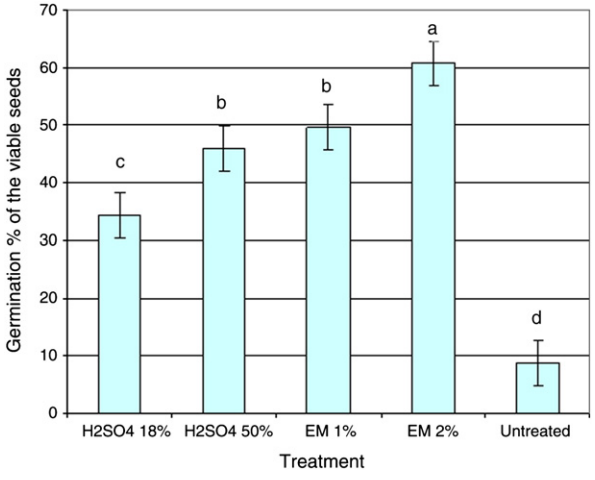


Fig. 2. Differences in germination percentage between treatments: (H_2SO_4 18%)= pre-treatment of seeds with 18% sulphuric acid for 20 min, (H_2SO_4 50%)= pre-treatment of seeds in 50% sulphuric acid for 20 min, (EM 1%)=pretreatment of seeds in 1% effective micro-organisms for twenty-four (24) h, (EM 2%)= pre-treatment of seeds in 2% effective micro-organisms (EM) for twenty-four (24) h and (Untreated)=soaking seeds in water only for 24 h to serve as the experiment's control. Bars with different letters are significantly different according to the least significant difference (LSD).

broke seed coats making it easy enough for the water and oxygen to be absorbed by the seed which later germinates. It is known that *H. procumbens* seed coats contain waxy materials that sometimes block water and oxygen entry to the seeds causing dormancy (Ellis et al., 1985; Kok, 1986). When this dormancy mechanism is broken seeds are germinated. As evident in the results of the current study, 50% concentration of sulphuric acid resulted in a higher (47%) germination rate than 18% concentration suggesting that the higher concentration (H_2SO_4 50%) was more corrosive to expose more seeds than the lower concentration (H_2SO_4 18%). This implies that in the wild, *H. procumbens* seeds will need extreme conditions in order to expose its seeds for germination.

The results of the present study support the findings of Zhang et al. (2006), Muhammad and Amusa (2003) and Rayachhetry et al. (1998). Though their results were on different species, the same principle underlying those results has been found to apply to *H. procumbens* as well. Contrary to the findings by Ernst et al. (1988) where no germination response was observed in seeds pre-treated with H_2SO_4, this experiment has found otherwise. This is attributed to the fact that the pre-treatment Ernst et al. (1988) made, was 100% concentrate of H_2SO_4, which could have been strongly corrosive as to damage even the embryos leading to no germination.

These results therefore denote that EM release beneficial compounds. These results imply that, like sulphuric acid, EM have the ability to breakdown the seed coat exposing the seed to germination. This is attributed to the fact that microbes in EM have the ability to breakdown organic matter (Higa, 1999) of which in this case may have disintegrated the seed coat. Similarly to H_2SO_4, higher concentrations (EM 2%) give higher germination (62%) percentages than lower concentrations (EM 1%). These high concentrations would however, indicate that potential suppliers of *H. procumbens* seedlings will need the solution in abundance in order to germinate more seedlings over a long period of time.

According to Baskin and Baskin (2004), temperature in nature plays a vital role in stimulating germination through high- or fluctuating habitat temperatures that seeds are exposed to after they are brought to the soil surface, such as after creation of a gap in the plant canopy. Considering the two facts (vegetation at the study sites and role of temperature in nature), the results of the current study done under fluctuating temperature (10 and 38 °C) imply that the only chance seeds would have to germinate in the wild would be when grasses dry-up (in seasons without rain) creating gaps in the plant canopy exposing seeds. With grass species, decimation by animals during dry seasons will lead to exposure of seeds. The presence of shrubs, that does not create many gaps in plant canopy even after drying of leaves, would therefore be a threat to seed germination since seeds will remain under shrub canopy cover for all seasons.

The reason why not all viable seeds germinated in the treatments could be attributed to that viability does not equal germination (Bonner and Gammage, 1967). This is attributed to the verity that was observed with viability testing of the current study.

4. Conclusions

EM have provided evident results to be one of the alternatives for germinating *H. procumbens* seeds. Sulphuric acid also enhances germination provided its concentration is not too high to damage the seed embryo as well. In order to contribute to a full understanding on the influence of EM and sulphuric acid on *H. procumbens*, further research on the establishment of *H. procumbens* seedlings pre-treated with EM or H_2SO_4 would help in making a concrete conclusion on its overall effect on the plant. This would be beneficial to all stakeholders of *H. procumbens* in sustainably managing this species.

Appendix A

Table 1. The number of seedlings that germinated according to treatments and attributes of treatments they germinated from. There were 34 seeds sown in 5 replicates for each treatment: H_2S0_4 18% treatment was the pretreatment of seeds in 18% sulphuric acid for twenty (20) minutes; H_2SO_4 50% was the pretreatment of seeds in 50% sulphuric acid for twenty (20) minutes, EM 1% was the pretreatment of seeds in 1% effective micro-organisms, EM 2% was the pretreatment of seeds in 2% effective micro-organisms, and control treatment with seeds untreated, only imbibed in water for 24 h before germination.

	H_2SO_4 18%	H_2SO_4 50%	EM 1%	EM 2%	Untreated (control)
Germination percentage (%) per treatment based on 82% viability	34.3	45.9	49.7	60.8	8.8
Germination per bag/per treatment					
Bag 1	5	8	8	15	4
Bag 2	9	9	9	10	1
Bag 3	8	7	11	11	1
Bag 4	6	10	9	12	3
Bag 5	7	13	11	14	0
Average number of seedlings germinated per bag/treatment	7	9.4	9.6	12.4	1.8
Stdev	1.58113883	2.302172887	1.341640786	2.073644135	1.643167673

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Edited by J Van Staden