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Note on Effect of Sulfuric Acid on Breaking Seed Dormancy of Least snout Bean (*Rynchosia minima* L.)[1]

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Abstract: Least snout bean (*Rynchosia minima* L.) is considered a promising rangeland legume fodder for marginal land in Sudan, but its seeds are associated with high dormancy (%). This study was carried out at the Laboratory of the Plant physiology, Department of Botany and Agricultural Biotechnology, Faculty of Agriculture, University of Khartoum to investigate the effect of sulfuric acid (H_2SO_4) on breaking seeds dormancy of least snout bean. The seeds were soaked in 0, 50, 70, and 100% H_2SO_4 concentrations for 30, 45, 60, 90 and 120 minutes. The seeds were germinated in petri dishes and incubated at room temperature for 10 days. The H_2SO_4 concentration and soaking period significantly ($P < 0.01$) increased seeds germination (%) and decreased mean germination time (MGT). The germination percentage was increased while MGT was decreased by increasing soaking period up to 60 min. The highest germination (%) and lowest MGT were obtained by soaking the seeds in 70 % H_2SO_4 for 60 minutes. The result would be useful for large-scale establishment of least snout bean in natural rangelands.

Keywords: Fodder plant; Germination rate; Dormancy; Chemical scarification.

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Rangelands are the main natural sources for feeding livestock in Sudan (Abdelrahim and Abdalla, 2015). However, they are highly variable and largely extend over arid, semi desert and savanna ecological regions (Abusuwar and Yahia 2010). The ecological regions are characterized by high rainfall variability, frequent droughts and extreme temperatures and edaphic conditions. Recently, most of the rangeland areas have been subjected to recurrent droughts and intensive overgrazing due to climate changes and tremendous increasing livestock population. Ultimately, a large number of range palatable plants species have extinct and replaced by unpalatable and invader plants species. Therefore, the productivity of the rangelands has been reduced and causing livestock feeding gaps. On the other hand a few forage legume plants species showed suitability for growing under harsh environments to fill livestock feeding gaps. Least snout bean (*Rhynchosia minima*) is a wild palatable legume species and distributed worldwide in a wide range of environments. It has ability to grow in semi-desert rangelands. Additionally it can improve soil fertility through nitrogen fixation process. The nutritional value of least snout bean is very high (Idris 2016). Least snout bean locally known as 'Adana', is considered as a promising rangeland fodder to fill livestock feed gap in Sudan. Generally, the seeds of least snout bean exhibit physical dormancy like many other legume plants species because of impermeable seed coat (Muller *et al*. 2017). Previous studies have shown the usefulness of some chemical treatments for breaking seed dormancy in different legumes species (Ali, *et al*. 2012; Naim, *et al*. 2015). Application of H_2SO_4 is the most widely used method for breaking seed dormancy by scratching the seed coat in some forage legumes species (Naim, *et al*. 2015). Based on the above-mentioned, the study aimed at determining the optimum H_2SO_4 concentration and soaking period for breaking seed dormancy in least snout bean to insure fast and uniform seed germination.

The study was conducted in the Laboratory of plant physiology, Department of Botany and Agricultural Biotechnology, Faculty of Agriculture, University of Khartoum, Sudan. The pods of *Rhynchosia minima* were collected from the demonstration farm, Faculty of Agriculture, University of Khartoum, Shambat, Sudan, in December 2019. The seeds were removed manually from the pods to reduce mechanical scarifications. Seeds were surface sterilized in sodium

hypochlorite (1% Na OCl) for 15 minutes and were washed three times with sterilized distilled water in a laminar air-flow cabinet. The (factorial) experiment was laid out in a completely randomized design with three replicates. The sterilized seeds were treated with H_2SO_4 concentrations of 50 %, 70% and 100 % for 0, 30, 45, 60, 90 and 120 minutes. The treated seeds were thoroughly rinsed in sterilized distilled water six times and then dried on top of filter paper for 24 hours on the laboratory bench. Non treated seeds were used as the control**.** Twenty of intact seeds were placed in petri dish containing two moistened filter papers (Whatman 9 cm) and sealed with clear plastic to prevent evaporation. All petri dishes were incubated at room temperature for 10 days and seeds were watered as needed with sterile distilled water. The number of germinated seeds was recorded daily. Seeds with a radicle length longer than 2-3 cm were considered as germinated seed and removed from the petri dishes. Imbibed, dead and dormant seeds were determined at the end of experiment. The following germination parameters were computed:

Germination (%) = (Number of germinated seeds/Total number of tested seeds) ×100

Mean time to germination (MGT) = $\sum (n*t)/ \sum n$
where t is time from the beginning of the germination test and n is the number of newly germinated seeds at time t. The collected data were subjected to analysis of variance (ANOVA) and mean separation among treatments was carried out by least significant difference (LSD) according to Gomez and Gomez (1984).

The effect of H_2SO_4, soaking period and their interaction on the seed germination (%) and mean germination time (MGT) of least snout bean is presented in Table 1. H_2SO_4, soaking period and their interaction had significant ($P< 0.01$) effects on the germination (%) and MGT of snout bean seeds. H_2SO_4 concentration significantly ($P < 0.05$) increased the seeds germination (%) and reduced MGT of Least snout bean compared to the control. Germination (%) reached a maximum level when seeds were treated with 50 % H_2SO_4 which is significantly higher than seeds that were treated with 70 and 100% H_2SO_4 concentrations. These findings are consistent with previous studies reported in different legumes species (Naim *et al*. 2015; Muller *et al*. 2017). The minimum seeds germination (13.3%) and the maximum MGT (3.27 days) were obtained in the control.

This result might be due to the presence of a strong hard seed coat inhibiting water absorption. The concentrations 70 and 100% H_2SO_4 showed the lowest and significant MGT (1.30 and 1.33 days, respectively). The mechanism of seed germination induced by H_2SO_4 is due to capability of H_2SO_4 to degrade hard seed coat, hence leading to water absorption and thus imbibition of seeds. This result agrees with previous studies by Imani *et al*. (2014). The soaking period significantly influenced seed germination. The germination (%) was increased while MGT was decreased by increasing soaking period of seeds in H_2SO_4. The highest seeds germination (%) was obtained at 60 minutes. It was observed that the germination (%) of seeds soaked for 90 and 120 minutes were lower than control by 36.7 % and 33.3 % respectively. The reduction of germination (%) might be due to damage of seeds caused by high concentrations of H_2SO_4. The soaking period of 90 and 120 minutes significantly ($P < 0.01$) exhibited the lowest MGT (0.71 and 0.77 day, respectively). This could be due to H_2SO_4 speeding up of seeds germination. Similar findings were obtained in Morong (Malavasi and Malavasi 2004), *Rhynchosia capitata* (Ali *et al*. 2012) and *Sabina vulgaris* seeds (Tanaka-Oda *et al*. 2009). Seed germination (%) was increased by increasing H_2SO_4 concentrations and soaking period until 60 minutes with a maximum value obtained when the seeds were soaked in 50%, 70% and 100 % H_2SO_4 for 60 minutes. The seeds soaked in 70% or higher H_2SO_4 concentrations for a period longer than 60 minutes lead to failure of germination (0 %) as the high H_2SO_4 concentrations might kill the seeds. The highest Germination (%) and lowest MGT were obtained for an interaction of 70 % H_2SO_4 and 60 minutes. These results are in agreement with previous studies conducted by Kher and Nataraj (2015) and Purohit *et al*. (2015). These results indicated that H_2SO_4 can speed up the germination rate because MGT is decreased. These findings indicated that H_2SO_4 is an effective method for releasing physical seed dormancy. Therefore, 70 % H_2SO_4 for soaking period of 60 minutes improved germination (%) and speeding up the germination of least snout bean seeds.

Table1. Effect of H_2SO_4, soaking period and their interaction on germination (%) and mean germination time (days) of Least snout bean seeds

Soaking period (min)	**Control**	**H_2SO_4 concentration (%)**			**Mean**
		50	**70**	**100**	
		Germination (%)			
Control	16.67 [d]				16.67 [b]
30		21.67 [d]	23.33 [cd]	18.33 [d]	21.11 [b]
45		96.67 [a]	100.0 [a]	98.33 [a]	98.33 [a]
60		100.0 [a]	100.0 [a]	100.0 [a]	100.0 [a]
90		31.67 [bc]	0.00	0.00	10.56 [c]
120		33.33 [b]	0.00	0.00	11.11 [c]
Mean	16.67 [c]	56.67 [a]	44.67 [b]	43.33 [b]	
LSD(P<0.05)_H_2SO_4		6.32			
LSD(P<0.05)_Soaking		5.00			
LSD(P<0.05)_Soaking*H_2SO_4		8.16			
		Mean Germination time (Days)			
Control	3.27				3.27 [d]
30		2.85 [b]	2.22 [ab]	2.33 [ab]	2.47 [c]
45		2.55 [ab]	2.27 [ab]	2.31 [ab]	2.38 [bc]
60		2.07 [a]	2.00 [a]	2.02 [a]	2.03 [b]
90		2.13 [a]	0.00	0.00	0.71 [a]
120		2.31 [a]	0.00	0.00	0.77 [a]
Mean	3.27 [c]	2.38 [b]	1.30 [a]	1.33 [a]	
LSD(P<0.05)_H_2SO_4		0.49			
LSD(P<0.05)_Soaking		0.39			
LSD(P<0.05)_Soaking*H_2SO_4		0.63			

Means of the germination % and means of germination time for H_2SO_4 soaking period and their interaction with the same letter(s) are not significantly different at $P < 0.05$ according to LSD

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أثر حمض الكبريتك على كسر الكمون في بذور الأضنة

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المستخلص: تعتبر الأضنة من أعلاف المراعي البقولية الواعدة للأراضي الهامشية فى السودان لكن بذورها ذات نسبة كمون عالية. أجريت هذه الدراسة بمعمل فسيولوجيا النبات، قسم النبات والتقانة الحيوية الزراعية، كلية الزراعة، جامعة الخرطوم لتقييم أثر حمض الكبريتيك على كسر كمون بذور الأضنة. نقعت بذور الأضنة في تراكيز مختلفة من حمض الكبريتيك (0، 50، 70، 100%) لفترات زمنية مختلفة (30، 45، 60، 90، 120 دقيقة). نبتت البذور فى أطباق بتري وحضنت فى درجة حرارة الغرفة لمدة 10 أيام. أظهرت النتائج أن معاملات حمض الكبريتيك ادت لزيادة معنوية ($P < 0.01$) فى نسبة ألانبات ومتوسط زمن الانبات. زادت نسبة أنبات البذور ونقص ومتوسط زمن الانبات بزيادة فترة النقع حتى 60 دقيقة. حصلت أعلى نسبة إنبات و ادنى ومتوسط زمن الانبات للبذور المنقوعه فى تركيز حمض الكبريتيك 70% لفترة 60 دقيقة. نتائج هذه الدراسة ستكون مفيدة لتاسيس نبات الأضنة على نطاق واسع في المراعي الطبيعية.