

Germination and Field Emergence of *Neorautanenia Brachypus* (Harms) for Livestock Survival in Zimbabwe

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ABSTRACT

There is a lack of information on establishment of *Neorautanenia brachypus* (Harms), a tuber legume critical for livestock survival. Studies were conducted in 2015 to determine the influence of different scarification treatments on establishment of *Neorautanenia brachypus* (Harms). Germination and emergence patterns were determined under laboratory and irrigated field conditions. *Neorautanenia brachypus* (Harms) seed attained peak germination and emergence of 45 % and 28 % under laboratory and field conditions, respectively. Mechanical and acid scarification had a significant ($P < 0.01$) effect on germination. Mechanical and acid scarified seed was first to germinate on day 5 after planting followed by control on day 6, KNO_3 submersion on day 8 and lastly prechilling and preheating treatments on day 12. The final germination was in the order mechanical (85 %) > control (45 %) > acid submersion (41 %) > KNO_3 submersion (26 %) > prechilling (19 %) > preheating (13 %). Under field conditions, emergence was significantly different ($p < 0.01$) with the highest for mechanical scarification (60%) followed by all four acid treatments (>50%) while the rest attained >30 %. This study shows that farmers can effectively establish *Neorautanenia brachypus* (Harms) in their fields using either mechanical or acid scarification. However, further study is required to ascertain tuber growth, shoots productivity and nutrient requirement.

Keywords: Germination, Field emergence, Scarification, *Neorautanenia brachypus* (Harms), Drought mitigation.

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Highlights of this paper

- *Neorautanenia brachypus* (Harms), is a recently discovered tuber legume critical for livestock survival.
- Germination and emergence patterns of *Neorautanenia brachypus* (Harms) was determined under laboratory and irrigated field conditions following various seed scarification methods.
- Highest germination and emergence were attained under mechanical and acid scarification.
- We advocate for mechanical scarification for establishing *Neorautanenia brachypus* (Harms) in farmers' fields.

1. INTRODUCTION

Cattle are a source of livelihood and fall-back mechanism for most smallholder farmers in Zimbabwe [1, 2]. They provide protein from meat and milk, draught power for tillage, manure for cropping and transport which are all vital for human survival [3]. Most smallholder farmers depend on natural grazing for feeding their cattle. However, due to an increase in deforestation and clearing of land for crop production, there is little space left for grazing purposes. Natural forage has become limited in both quantity and quality due to overgrazing. The limited range of available feeding strategies particularly in the dry regions of most smallholder farming systems in Sub-Saharan Africa is the main problem affecting livestock survival. Depending on rainfall season quality and crop management, cereal crop residues can be useful as a feeding strategy during the dry season. Supplementary feeding could boost cattle production but a few resource endowed farmers can afford to procure them [4]. During extreme droughts, cattle die in large numbers due to lack of feed and water in addition to outbreaks of diseases. Extreme climatic conditions are predicted to become more frequent as a result of climate change [5] with dry conditions set to increase in southern Africa. As such, there is need to find ways to improve quality of natural pastures and searching for alternatives to ensure survival of the cattle.

The introduction of forage grasses and pasture legumes in smallholder farming systems has been cited as a beneficial option of improving cattle survival [6]. *Cynodon nlemfluensis* and *Pennisetum purpureum* have shown a positive impact in terms of improving the carrying capacity and production unit [6, 7]. Due to their quality protein addition, legumes such as *Lablab purpureus* and *Stylosanthes scabra* have also shown great potential to improve livestock feed availability and quality [8]. Despite the biological nitrogen fixation potential, pasture legumes have not been fully utilized in smallholder farming systems in Zimbabwe due to soil phosphorus deficiency, inoculation requirements, availability and accessibility of seed due to high cost [9]. Although cattle production is most suitable in the Lowveld of Zimbabwe; there are few forage legumes like *Chamaecrista rotundifolia* and *Macroptilium atropurpureum* which are adaptable in areas that receive < 800 mm rainfall per annum [10]. As a result of climate change, the region is predicted to become warmer, drier and rainfall distribution skewed within a season, which is likely to have an impact on crop residue production, natural forage and pasture legume establishment.

Similar to the over 36 indigenous legumes species identified for soil fertility restoration using indifallow systems [11-14] an indigenous tuber legume named *Neorautanenia brachypus* (Harms) has been identified by farmers for utilization in Lowveld of Zimbabwe [15]. Further study by Nyarumbu, et al. [16] discovered the existence of different species of the species basing on the leaf morphology and genetic characteristics [16] yet domestication effort remains limited. Such discovery and advancement have brought some hope to hard-pressed local farmers with potential fallback strategy during severe droughts and dry season. *Neorautanenia brachypus* (Harms) tubers have been seen to provide adequate nutrients, water and medicinal benefits [15] to cattle. It has also been shown that the tuber has different colours and cattle mostly prefer the white tubers that are soft and juicy [16]. Many other

indigenous tuber legumes remain undomesticated and underutilized yet contain a lot of protein compared to the conventional soybean and groundnuts [17]. For example, *Tylosema esculentum* L. (Marana bean) is a tuber legume that has received little attention. However, the capacity of *Neorautanenia brachypus* (Harms) to establish on various soils, restore and maintain cattle health status requires full attention for harnessing into the cropping system [15, 18] and also elicits a lot of scientific interest. Tapping into this naturalised indigenous legume, however, requires information on propagation and field management. Building an understanding of *Neorautanenia brachypus* (Harms) establishment might be a starting point in the domestication of the tuber legume into farming systems of Southern Africa for livestock survival. There is a need to understand and appreciate its agronomy starting with propagation. The objective of the study was to investigate seed germination and the emergence of *Neorautanenia brachypus* (Harms) under laboratory and field conditions.

2. MATERIALS AND METHODS

2.1. Seed Harvesting

Neorautanenia brachypus (Harms) seed was collected from the south-east Lowveld of Zimbabwe in Chikombedzi (18°05'S, 29°27'E) resettlement area Figure 1 by farmers from natural stands in March 2015. Chikombedzi is 800 km southeast of Harare and is found in agro-ecological region natural region V, which receives average rainfall below 450mm per annum between November and March. The maximum temperature in that region is about 35°C and the minimum temperature is about 15°C. *Neorautanenia brachypus* (Harms) is found in the farmers' fields and in the surrounding woodlands where it grows naturally but has poor seeding ability. The seeds were randomly collected from over fifty plants by handpicking mature pods with rattling seed inside within the Chikombedzi ward. The pods were threshed and the seed was collected following winnowing and then mixed to make one homogenous composite sample. Seed harvesting and post-harvesting processing ensured that only mature seed was utilized in this study.

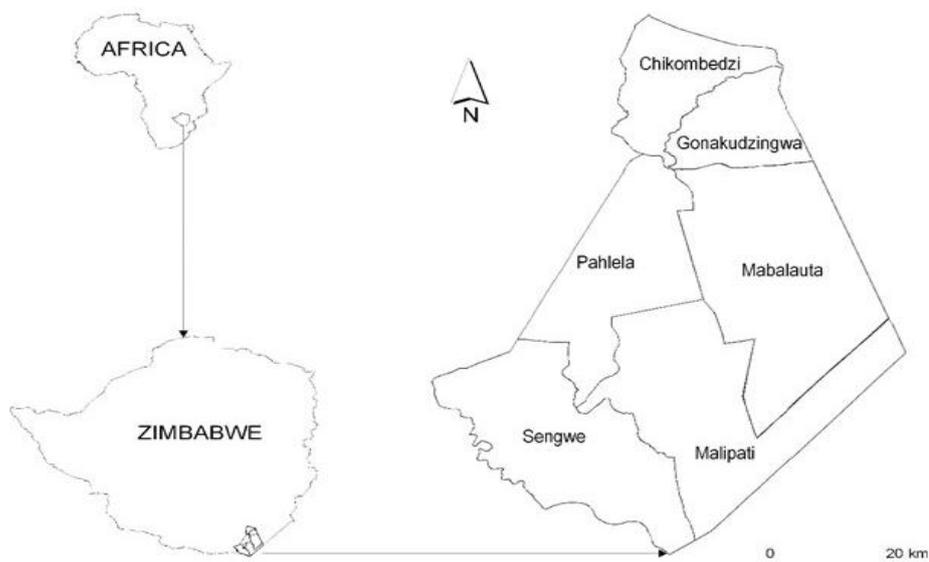


Figure-1. A map indicating the location of chikombedzi in the southeastern part of Zimbabwe on the edges of gonarezhou national park in Masvingo province.

Source: Poshiwa [19].

2.2. Seed Characterisation

Two replicates of 0.5 kg of *Neorautanenia brachypus* (Harms) seed was ground in a Wiley Mill to pass through a 1 mm sieve. Various proximate analyses were then done in replication from the two ground samples. Moisture content (MC) was determined gravimetrically by oven drying for 17 hours at 103 °C (to obtain dry weight (DW))

[20] and expressed on a fresh weight (%FW) basis by the equation; $\%MC = (FW-DW)/FW * 100$. Percentage ash was determined by burning the seed sample in a muffle furnace for 17 hours at 250 °C. Total N analysis was done by the Kjeldhal digestion method [21] and crude protein was obtained by multiplying the seed N concentration by a constant factor (6.25) [22]. Total fat was analysed by subjecting the sample to continuous extraction using soxhlet extractor with the solvent being petroleum ether [22]. The crude fibre was determined after digesting the sample with 1.25 % H₂SO₄ and 1.25 % NaOH solution [22]. The percentage of carbohydrate was determined by subtracting the percentages of all proximate analysis from 100%.

2.3. Preliminary Germination Test

Four replicates of fifty seeds were randomly sampled from the composite sample and evenly distributed on plastic trays measuring 20 cm by 15 cm by 3 cm which was filled with a 2 cm layer of sand from the bottom. Before using the sand medium was thoroughly washed with acid, followed by running tap water, sterilized at 200 °C in Memmert sterilizer Beschickenh-loading model 100-800 for four hours and cooled naturally [20]. After evenly distributing the seed, four trays were filled with sand to cover the whole seed at 1 cm depth and moistened with tap water to allow favorable moisture content for germination. The trays were covered with polythene or plastic bags to reduce moisture losses by evaporation during the test. The trays were laid out in constant room temperature at 25 °C under two daylight type fluorescence lamps each of 40w. The experiment was laid out as a Randomized Complete Block Design with four replicates. Blocking was based on light intensity. Germinating seeds were counted by destructive sampling, with all the germinated seeds removed on each day of counting. A duly germinated seed was classified based on the appearance of essential structures (primary root, shoot system, and primary leaf) from the embryo. The essential structures should be intact or showing acceptable defects and indicating the ability to develop into a normal plant under favourable soil conditions [20].

2.4. Determination of *Neorautanenia Brachypus* (Harms) Germination Following Scarification Under Laboratory Conditions

Sub-samples of 50 *Neorautanenia brachypus* (Harms) seeds per tray were subjected to different scarification methods as per treatment requirements. The following treatments were used:

- i. Control (untreated seed).
- ii. Mechanical.
- iii. Sulphuric acid (H₂SO₄) submersion for 2 minutes.
- iv. Preheat at 35 °C for 7 days.
- v. Prechilling at 5-10°C for 7 days.
- vi. 2% KNO₃ submersion.

The control had an unscarified matured seed of good quality. Mechanical treatment involved rubbing the seed on a rough surface until the coat and part of the cotyledon were removed while avoiding damage to the hilum and radicle. Sulphuric acid treatment involved submersion of seeds in 98% concentrated H₂SO₄ for 2 minutes at room temperature. The mixture was occasionally stirred using a glass rod followed immediately by rinsing under running water for one minute and air-dried [23, 24]. Preheating treatment involved placing seed in ventilated ovens set at 35 °C for 7 days before sowing [25]. Prechilling involved placing the seed on top of filter paper with a moisture towel underneath in glass Petri dishes in a refrigerator at 5-10 °C for 7 days [20]. Potassium nitrate treatment involved submersion of seeds in 2% concentrated KNO₃ for 2 minutes at room temperature [20]. The seeds were then subjected to a germination test similar to that described for the preliminary test above.

2.5. Determination of *Neorautanenia Brachypus* (Harms) field Emergence Following Various Scarifications

The study was conducted at Marondera University of Agricultural Sciences and Technology (MUA) located 40 km south of Marondera town within Natural Region IIa. The area experiences a sub-tropical climate with average summer temperatures of 25 °C and annual precipitation between 800 – 1 000 mm year⁻¹ following unimodal rainfall pattern on setting in October and ending in April. Before the planting, the seed of *Neorautanenia brachypus* (Harms) was subjected to the following scarification treatments:

- i. Control (untreated seed).
- ii. Mechanical.
- iii. Sulphuric acid (H₂SO₄) submersion for 1 minute.
- iv. Sulphuric acid (H₂SO₄) submersion for 2 minutes.
- v. Sulphuric acid (H₂SO₄) submersion for 4 minutes.
- vi. Sulphuric acid (H₂SO₄) submersion for 6 minutes.
- vii. Preheat at 35 °C for 1 day.
- viii. Preheat at 35 °C for 2 days.
- ix. Preheat at 35 °C for 3 days.
- x. Preheat at 35 °C for 4 days.
- xi. Preheat at 35 °C for 5 days.
- xii. Preheat at 35 °C for 6 days.
- xiii. Preheat at 35 °C for 7 days.
- xiv. Prechilling at 5-10 °C for 1 day.
- xv. Prechilling at 5-10 °C for 2 days.
- xvi. Prechilling at 5-10 °C for 3 days.
- xvii. Prechilling at 5-10 °C for 4 days.
- xviii. Prechilling at 5-10 °C for 5 days.
- xix. Prechilling at 5-10 °C for 6 days.
- xx. Prechilling at 5-10 °C for 7 days.

The process for acid scarification, preheating and prechilling was similar to that for laboratory germination. For the field trial, time under different scarification methods was factored in to avoid missing out on the best possible options which farmers can utilize. The site was plowed using a tractor and conditioned to a fine tilth. The soils are coarse-grained sandy loam derived from the granite parent material. A composite soil sample (0-30 cm) was taken from the site analysis before planting and likewise, soil samples were also collected in Chikombedzi at Zanamwe farm. A randomized complete block design (RCBD) with three replicates per treatment was effected on plots measuring 1 m by 0.5 m. Thirty seeds were planted at 2 cm depth in 5 rows with six seed per row with an in-row spacing of 15 cm. Watering was done using a horse pipe twice a day to ensure adequate moisture for germination and emergence. Emerging plants were counted at two days interval from the 1st emergence over time in the plots. A duly emerged seed was classified based on the appearance of the shoot system and primary leaf from the seed embryo.

3 RESULTS

3.1. Germination of *Neorautanenia Brachypus* (Harms) Seed Under Laboratory Conditions

Neorautanenia brachypus (Harms) seed is seen to be rich in nutrients as indicated by high crude protein and fibre which are both > 31 % while carbohydrate is ~18 %. The seed also has high ash content aligning with high calcium

and sodium Table 1. Preliminary germination test indicated that 23 % of the seeds germinated 13 days after planting and reached a peak of 44 % 17 days after planting Figure 2.

Table-1. Chemical characteristic of *Neorautanenia brachypus* (Harms) seed.

Material	Moisture	Ash	Crude protein	Fat	Crude fibre	Carbohydrate	N	P	Ca	NaCl
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Seed	6.83	4.56	31.04	11.3	32.47	18.36	4.97	0.45	0.34	0.1

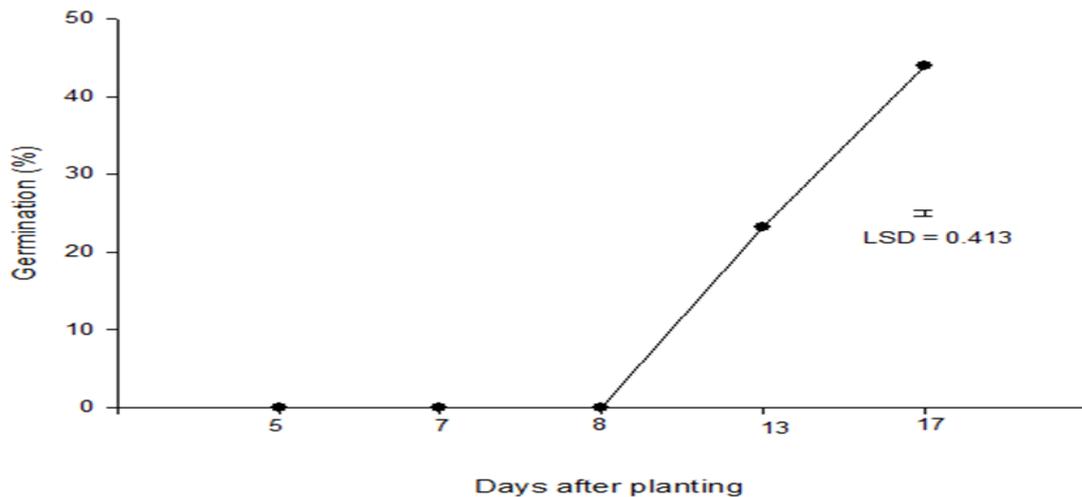


Figure-2. Germination of *Neorautanenia brachypus* (Harms) species on sandy soil under laboratory conditions.

3.2. The Effect of Scarification of Germination on *Neorautanenia brachypus* (Harms) Seed Under Laboratory Conditions

Scarification had a significant ($P < 0.01$) effect on the germination of *Neorautanenia brachypus* (Harms) seed. Mechanically and acid scarified seed were the first to germinate on the 5th day after planting followed by control on the 6th day, KNO_3 submersion on the 8th and lastly prechilling and preheating treatments on day 12 Figure 3. Mechanically scarified seed reached 81 % germination on the 6th day and peaked to 85 % on the 12th day and was significantly higher than that for other treatments Figure 3. Though the control germinated earlier (day 6) than the acid scarified seed, percent germination was 9 % higher than acid-treated seed on day 8. However, the control later peaked to 45% after surpassing the acid treatment on the 10th day Figure 3. Between 8 and 12 days, germination percent of KNO_3 submersion treatment was 8 % and increased to 26 % on day 13 through to 18. The KNO_3 submersed seeds had a higher germination rate than the control 18 days after planting. The final germination was in the order of mechanical (85%) > control (45 %) > acid submersion for 2 minutes (41 %) > KNO_3 submersion for 2 minutes (26 %) > Prechilling at 5-10 °C for 7 days (19 %) > preheating at 35 °C for 7 days (13 %) Figure 3.

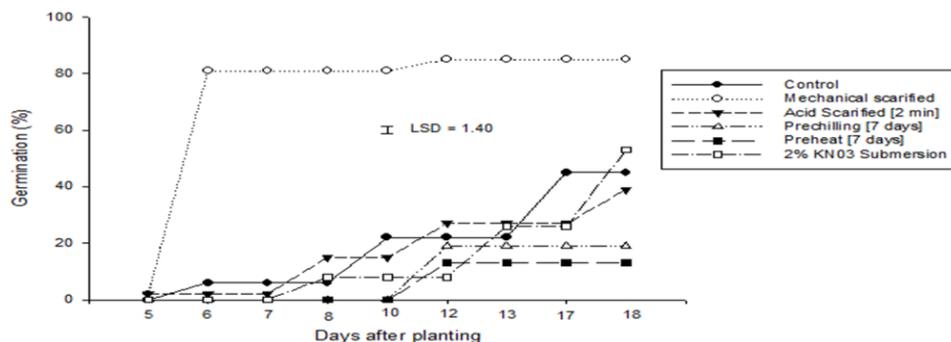


Figure-3. Cumulative germination of *neorautanenia brachypus* (Harms) seed following different scarification treatments under laboratory conditions.

3.3. Physicochemical Characteristics of Soils

The soil at Marondera University of Agricultural Sciences and Technology site was medium-textured soil and generally fertile (high nitrogen and available phosphorus) indicating good management with high fertilizer utilization Table 2 as compared to Zanamwe farm in Chikombedzi Table 2. Furthermore, Zanamwe soil was alkaline which was due to the high exchangeable calcium and magnesium in the soil Table 2.

Table-2. Chemical characteristics of soils at 0-0.3 m depth from Chikombedzi and trial site at the MUAST farm in the dry season of 2015.

Site	Texture range + FAO class	pH (CaCl ₂)	‡Initial-N	Incubated-N	§Available-P	¶Exch. K	#Exch. Ca	##Exch. Mg
			mg kg ⁻¹			cmol(+)kg ⁻¹		
Land 1	mgSL	6.9	9	17	28	1.07	40	13
Land 2	mgLS	6.0	16	50	86	0.44	4.49	1.01

Land 1 is Zanamwe farm in Chikombedzi and Land 2 is Trial Site at MUAST; +fg=fine-grained; mg=medium-grained; SC=Sandy-clay; SCL=Sandy-clay-loam
 General fertility range interpretation: adapted from Tanner and Grant (1963); Grant (1981). ‡Mineral-N measured after a 14-day incubation of soil at field capacity and at 35 °C: <20=very low; 20-30=low; 30-40=medium; >40=high. §Available-P (resin-extracted): <7=very low; 7-15=low; 15-30=medium; 30-50=high.
 ¶Exchangeable-K: <0.15=very low; 0.15-0.3=low; 0.3-0.5=medium; >0.5=high. #Exchangeable-Ca: <5=very low; 5-10=low to medium; >10=high
 ##Exchangeable-Mg: <0.1=very low; 0.1-0.2=low to medium; >0.2=high.

3.4. The Effect of Scarification of Emergence on *Neorautanenia Brachypus* (Harms) Seed Under Field Conditions

Scarification and field conditions had a significant effect on the emergence profile of *Neorautanenia brachypus* (Harms). Generally, emergence started 13 days after planting with 5 noticeably superior treatments with an initial emergence ranging from 8 to 14 %. The other 15 treatments had <4 % emergence during the same period. The superior treatment included mechanical scarification and all four acid treatments Figure 4. All five superior treatments showed a huge leap in emergence from 13th day to 17th day which further separated the acid scarified treatment into two groups Figure 4. Among the 15 low performing scarification treatments, prechilling for 7 days had relative high emergence between 13 and 17 days but was surpassed by the control at the end Figure 4. Mechanical scarification had the highest emergence over time reaching a maximum of 60%. Seed submerged in acid for 1, 2, 4 and 6 minutes reached maximum emergence of 57 %, 58 %, 52 %, and 58 %, respectively but did not significantly differ. However, regression analysis indicated that acid scarification for 2 minutes had the largest slope of regression (4.40) than mechanical scarification and other acid treatments Figure 5. The control, preheating and prechilling treatments had significantly ($P<0.01$) lower emergence than the acid treatments though the control had a generally higher emergence. Overall, the lowest emergence was observed in the treatment preheated for 7 days (8 %) while the control attained 28 % emergence Figure 4.

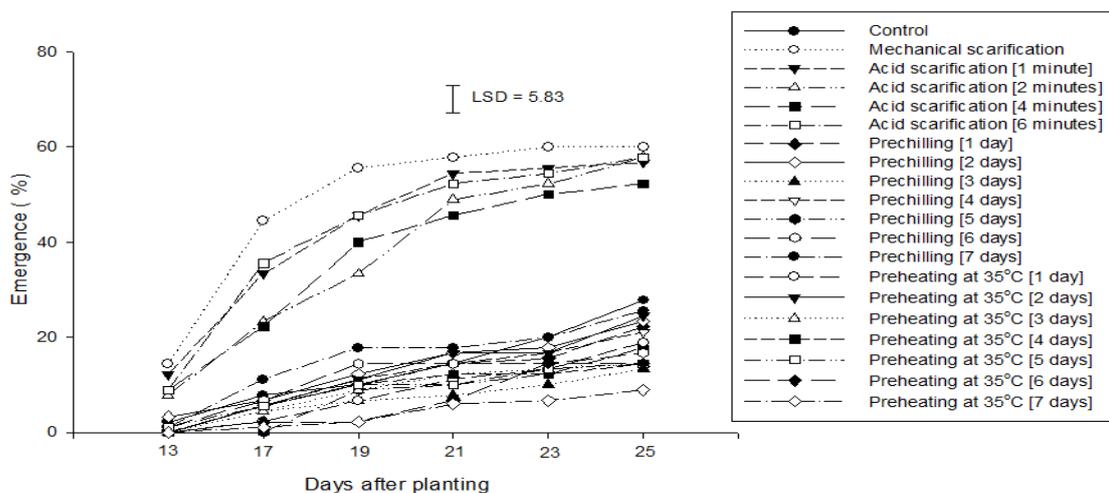


Figure-4. The emergency of *Neorautanenia brachypus* (Harms) following different scarification treatments under field conditions.

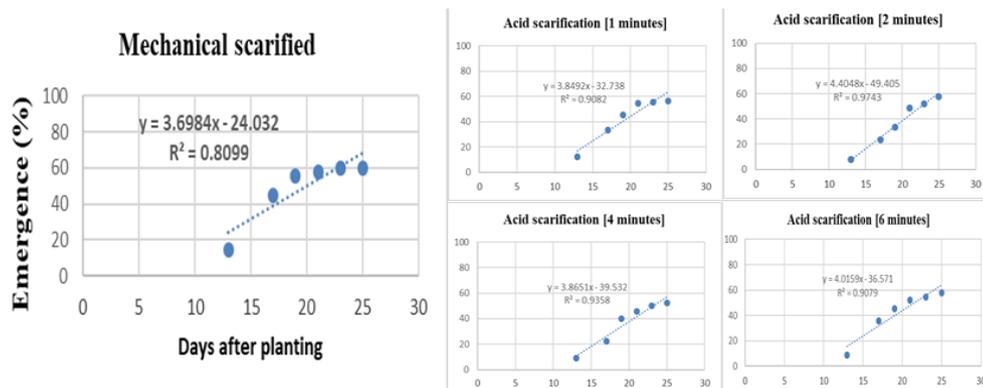


Figure-5. Regression curves for selected treatments under field conditions.

4 DISCUSSION

4.1. Germination of Seed

Neorautanenia brachypus (Harms) seed attained peak germination of 44% and 45% in the preliminary and scarification tests respectively. This is not what would have been expected considering the high nutrient content in the seed Table 1. Seed with high nutrient content is likely to support germination when all germination requirements are optimum [24]. *Neorautanenia brachypus* (Harms) can be considered to produce large seed with high food reserves. Large seed tends to produce seedling with great vigor and has a competitive advantage over pests and diseases. Large seeds are considered to have higher germination percentages [26-28] and reduced germination time [29] compared to smaller seeds due to the large nutrient reserves. This notion is also supported by research done by Arunachalam, et al. [30] and Tauro, et al. [24] on tree species. They attributed good seed germination and emergence to the large food reserves in the larger seeds. Observations with *Neorautanenia brachypus* (Harms), however, were contrasting. Though adequate moisture, air, and warm temperature were provided in the laboratory to initiate the germination process, the seed required approximately two weeks for germination to commence indicating restriction to the germination process. Consistently in the second run, seed managed to reach peak germination of 45 %. This evidence suggests the presence of some seed dormancy probably in the form of a hard seed testa/coat. Though seed size and nutrient content can be indicators of seed quality and germination other factors such as time of seed collection [31] and genetic makeup [32] can contribute to seed germination and vigor.

4.2. The Influence of Scarification of Seed Germination Under Laboratory Conditions

Legume seed dormancy is an inherited trait attributed to the seed coat which has cell walls that are thick and covered by a waxy cuticle [33]. This coat makes the seed impermeable to water thus affecting germination and removal of the seed coat becomes important. Mechanical scarification reduced the time to germination and increased germination percent of *Neorautanenia brachypus* (Harms) Figure 3 which was indicative of the removal of physical dormancy. Mechanical and acid scarification methods involved the removal of seed coat which promoted faster and easier water entry into the seed embryo subsequently activating enzymes responsible for hydrolyzing stored food for the germination process [34]. Acid scarification in the laboratory did not produce results significantly different from the control. The short time used for acid scarification might not have sufficiently destroyed the seed coat to promote the movement of air and water into the seed. The low germination percentage recorded for the acid treatment indicates that two minutes of seed-acid contact was not adequate to remove the seed coat. It has been reported that submersion of seed in acid for 10 minutes [35] 15 minutes [36] 20, 25 and 30 minutes [37] increased germination percentages in *Piptadenia moniliformis* seed. The germination of *Macrotyloma daltonii* and several indigenous legumes species were improved by acid scarification [14, 25]. However, Garcia, et

al. [38] reported that immersion of seed in sulphuric acid for more than 15 minutes could reduce germination due to seed damage. Despite the low germination for the greater part of the monitoring period, seed treated with 2 % KNO₃ surpassed the control reaching 26 % at the end of the test. This suggests a time lag for breaking dormancy. Prechilling had higher germination percentage than preheating indicating that the prechilling process might have activated some biochemical reaction in the seed to allow for better germination. During prechilling, the towel and filter paper were moist, slowly allowing seed imbibitions which could not happen during preheating due to the absence of water. The low germination percentage attained following preheating at 35 °C for 7 days might be attributed to denaturing of the enzymes and destruction of the food reserves in the seed.

4.3. The Influence of Scarification on Seed Germination Under Field Conditions

The high emergence attained following mechanical and acid scarification confirms that *Neorautanenia brachypus* (Harms) has physical dormancy. Such high emergence from both mechanical and acid-treated seed showed that they are effective methods for the establishment of *Neorautanenia brachypus* (Harms). The high emergency shown by all the four acid treatments supports the idea of modifying the treatments when transferring from laboratory experiments to field conditions. It is clear from this study that acid scarification and mechanical removal of the testa greatly increased germination but regression analysis indicated that acid scarification for two minutes was the best options for removing the dormancy. The results from this study agree very well with Neme [39] who observed that sulphuric acid effectively increase seed germination in perennial soyabean to 100 % as compared to 7-24 % without scarification. In this study, acid scarification had a peak germination percent nearly double that for the control. Mechanical scarification works well on the seed with a water-impermeable seed coat [40] and thus we can assume that *Neorautanenia brachypus* (Harms) seed dormancy is due to a hard seed coat. Immersion in hot water, scarification with sandpaper and chemical scarification has been used successfully to break physical dormancy of leguminous seed [41]. Farmers are likely to utilize mechanical scarification than acid scarification which requires financial resources and skill to use. Mechanical scarification methods for bulk seed treatment are required. The general reduction in emergence under field conditions, when compared to germination in the laboratory, suggests there are a lot of soil and environmental factors in play that determines the final emergence. It is, therefore, imperative to utilize results from field studies if accurate recommendations are to be made. All prechilling and preheating treatments attained similar or lower final emergence indicating that they are not effective in breaking seed dormancy of *Neorautanenia brachypus* (Harms). However, exposing the seed to prechilling conditions for seven days showed high emergence during the later stages suggesting that a longer period of prechilling could be beneficial in establishment *Neorautanenia brachypus* (Harms). Future studies may need to determine the optimum prechilling period. Consistent with the laboratory results, preheating for seven days had a detrimental effect on the emergence of *Neorautanenia brachypus* (Harms) seed.

5. CONCLUSIONS

The seed of *Neorautanenia brachypus* (Harms) attained peak germination of 45 % and peak emergence of 28 % under laboratory and field conditions respectively. Mechanical scarification and acid scarification were superior in reducing the physical dormancy of *Neorautanenia brachypus* (Harms) and also reducing the time to germination and emergence. Mechanical scarification increased germination by 40 % to peak at 85 % within 12 days of planting while it increased field emergence to 60 %. Acid scarification treatment also showed positive results by increasing germination and field emergence. From the regression results, farmers can use 2 minutes of acid scarification to save on labour and time. Prechilling for < 7 days and all preheating treatments had no positive effect on

germination and emergence of *Neorautanenia brachypus* (Harms) seed. The results indicated that farmers can effectively establish *Neorautanenia brachypus* (Harms) in their fields using either mechanical or acid scarification seed. This knowledge of germination and emergence of *Neorautanenia brachypus* (Harms) can contribute to the survival of livestock in driest parts of Zimbabwe and similar agro-ecologies in Sub-Saharan Africa if the species is domesticated.

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