Original Research Article

Seed pre-treatment and early growth performance of *Piliostigma thonningii* (Schum.) Milne-Redhead under nitrogen amendment in Old Oyo National Park, Nigeria

Abstract

Survival of wildlife in protected areas is dependent on innate attributes and response of vegetation to climate change. *Piliostigma thonningii* holds promise for wildlife conservation in Old Oyo National Park, but its population is threatened by physical dormancy. There is dearth of information on long-term benefits of its seed pretreatment and early growth response to nitrogen fertilizer. This study assessed effects of acid scarification on germination and early growth of *P. thonningii* under different nitrogen application rates. Seeds harvested from three purposively selected seasonal wetlands in Old Oyo National Park (OONP) were treated with 1N H_2SO_4 at durations ranging from 0 – 120 minutes in three replicates. Germinated seeds were enumerated over a 14-day period. Top soils from the wetlands were randomly sampled using soil auger in three replicates for physical and chemical analyses, and for early growth study with Urea fertilizer at rates 0, 50 and 100 kg/ha in experimental pots in completely randomized design. Growth and dry matter were assessed using ANOVA at *P.05*. Germination commenced in control treatments at 14 days after plating (DAP) and was low (13.3%). Germination progressively increased in acid treatment. At 5 DAP for soaking for 30 minutes gave 46.67%; while it was 96.67% at 40 minutes at 14 DAP. Higher soaking times gave 100% germination by 14 DAP, but with low growth vigour. The response of *P. thonningii* to Urea application was significantly higher in number of leaves at 12 weeks than in other rates. Other growth parameters are not significantly different at all rates. The biomass yield of *P. thonningii* was 1.98g±0.21and 2.6g±0.36 root and shoot dry weights at 6 weeks after sowing (WAS); and 7.3g±0.04 root and 6.4g±0.95 shoot dry weights at 12 WAS. *Piliostigma thonningii* can restore and improve wildlife abundance to mitigate climate change effects in the Park.

Keywords: Seed dormancy, Climate change, *Piliostigma thoninngii*, Old Oyo National Park, urea fertilizer, early seedling establishment.

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Introduction

Conservation of wild ungulates is closely linked to availability habitat for browse and forage species in their habitats (Gedir *et al.*, 2015; Zeller *et al.*, 2021). In the case of pronghorn, a loss of high-quality habitat and areas of connectivity with projected climate change was reported by Zeller *et al.* (2021), and which may negatively affect their populations and wellbeing throughout their natural range. Browse and forage species provide nutrients and ensure good wellbeing of the ruminants for survival and productivity. Plants in the wild are dependent on interplay of environmental factors and their gradients to thrive. The most important being climatic and edaphic factors, as well as occasional anthropogenic interventions in the form of bush clearing using fire, chemicals or other physical means. Climate of regions of the world is rapidly changing, especially in tropical areas, causing changes in vegetation (Raiho *et al.* 2022).

Climate is implicated in influencing progressive replacement of browse plants with grasses that are well adapted to drier environments. This is often heightened in savanna ecosystems which are already dry. In the wild, especially in woodlands, fire and browsing by animals affect resprouting of browse species (O'Çonnor *et al.,* 2020). This is a case of circularity, but which must be addressed in favour of establishing more browse species, especially in the wooded savanna of tropical regions. This could be achieved through enrichment planting (Forbes *et al.,* 2020), in tandem with proffering solution to the fundamental environmental and biotic causes of species erosion.

Plants require some minerals such as nitrogen, phosphorus and potassium for growth and development. Major changes in plant species composition and plant life forms are associated with gradients in soil fertility (Keddy, 2000). The role of fertility in controlling vegetation composition has been studied by many wetland ecologists. Verhoeven *et al* (1996) through their experiment on biomass response to fertilization indicated that some limiting factors affect plants in the seven habitat types used for the experiment. They showed that nitrogen and potassium limit plant performance twice and thrice respectively; and that N+K limited the performance four times in wet grassland. Phosphorus limited it thrice in wet heathland as opposed to a no-effect of N and K. Fens and dunes were however observed to be liable to either nitrogen or phosphorus limitation.

A review of 45 studies on fertilization in seven types of herbaceous mires by Koerselman and Meuleman (1996) revealed an almost even split between nitrogen splited sites and phosphorus-limited sites. Likewise, the work of Barko and Smart (1979) on substrate gradients using wetland plants revealed N levels to range from 0.3 g/kg in sand to 5.2 g/kg in silty clay, while P levels range from <0.05 g/kg to 1.65 g/kg in sand to silty clay respectively. An extended implication of plant nutrient limitation is a comparable nutrient deficiency in animals utilizing such plants; because, plants are a basic source of these nutrients to animals. This means that status of available nutrient in wetlands determines to a large extent, the nutritional value of wetland plants and animal health and residency.

Since plants are very economical in their use of nitrogen, at very best concentrating it in seeds, pollens or cambium, animals may be able to harvest a food source that is about 5% nitrogen dry weight (White, 1993); therefore, suggesting that nitrogen, not energy is the limiting resource for animal communities (White, 1993; Keddy, 2000). It is important, however, to note that while biomass and plant species composition may change with soil fertility, the per gram availability of nitrogen to animals may not because there is no difference in N concentration between plants typical of habitats with high and low fertility (Chapin, 1980; Keddy, 2000).

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Thus, the fate of many wild ungulates rests on potentials of protected spaces to provide, not only refuge, but also enough food resources. The ability of these protected spaces to perform this ecological function depends on proper management.

Old Oyo National Park (OONP), as one of eight national parks in Nigeria, houses a wide array of wild fauna species which are sustained throughout the year by pockets of wetland vegetation provided by the wetlands around Rivers Ogun and its tributaries that traverse the Park. The environmental importance of Old Oyo National Park was demonstrated in the recognition of its status as a National Park in 1988, though it was gazetted on 10th March 1973. In the face of Local extinction of plant species which has been widespread in Africa. The first task in conservation therefore is to identify species that are at risk (Ectringhan, 1979). In the case of OONP, *Piliostigma thonningii* is an important browse species that has been identified (Olubode (2007); Olubode *et. al.*, 2009; Olubode and Awodoyin, 2012).

Woodward (1987) suggests that the temporal dynamics of species are dependent upon the relation between scale of environmental variation and the time scale of organismal response. Therefore, a fast growing highly productive plant would be desireable for conservation of wildlife in protected areas. Old Oyo National Park is located in southern guinea savanna in Nigeria. Keay (1959) outlined four savanna types based on climatic and conventional zonation as Transition savanna, Guinea savanna, Sudan savanna and Sahel short grassland. He characterized the Transition savanna with over 200 rainy days, 0.75 to 1.0 precipitation/evapotranspiration ratio; being burnt annually; highly disturbed by man and typically a mosaic of agricultural land; with forest remnants and grassland.

The herbaceous components of the Park include, *Andropogon tectorum* and *Andropogon gayanus* which are richer in proximate contents than *Hyparrhenia involucrate* and *Hyparrhenia rufa* (Olubode *et al.*, 2009). Being located in the Guinea savanna, it I s characterized with a length of rainy days of 150 to 200 days. Precipitation/evapotranspiration ratio of 0.40 to 0.75, and as being burnt annually (Keay, 1959; Olubode *et al*., 2009). *Piliostigma thonningii, Detarium microcarpum, Terminalia spp. Combretum spp., Burkea african, Vitellaria paradoxa, Afzelia africana, Daniellia oliveri,* and *Isobelinia spp* are abundant in OONP (Olubode *et al*., 2009; Olubode and Awodoyin, 2012).

P. thonningii, a well-known forage species (FAO, 1999) was fast disappearing from OONP (Olubode, 2007, 2009). The result of FAO's (1999) analyses of *Piliostigma thonningii* as an important browse plant for games and cattle showed that fresh leaves of the plant contained relatively higher amounts of percent crude protein, crude fibre and extractable ether (crude fat) than those in pods, and that these vary among the three countries (Niger, Zimbabwe and Nigeria) considered. However, the NFE content was higher in pods than in leaves. This explained why it is a preferred browse plant. It is suspected that prolonged dormancy offered by hard seed coat contributes to its unavailability on one hand and the depletion of nitrogen content in soil by annual burning affects the early growth of few germinated ones on the other hand (Olubode, 2007). Thus, only few mature individuals remain to replenish the Park.

Seed dormancy is a natural mechanism and an innate seed property that defines the environmental conditions in which the seed is able to germinate (Finch-Savage and Leubner, 2006). Plants use seed dormancy to regulate regulates its population so as to avoid unfavourable biotic and abiotic environmental conditions, or to ensure continued existence of its kind without wasting its perenating materials. It is beneficial in legume domestication ad and in the wild (Soltani *et al.,* 2021), where environmental conditions can be has and variable. Dormancy may be due to impermeability of seed coat to water and gases caused by deposition of suberin, lignin and cutin in the responsible membrane or across the micropylar opening as in most legumes; hinderance of seedling growth as a result of mechanical

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resistance by seed coats; embryo or physiological dormancy caused by physiological immaturity of embryo as in most grasses; the presence of growth inhibiting chemicals (Copeland, 1976; Shu *et al.*, 2016; Soltani, 2021). Seed dormancy, because it spreads seedling recruitment over a period of time in an area, is termed "temporal dispersal" (Radosevich and Holt, 1984).

Gashaw and Michelsen (2002) reported that seeds of *Piliostigma thonningii* responded to heat treatment of 20°C up to 200°C for 5 minutes in an experiment to simulate heat shock caused by savanna bush burning on seeds found in upper soil layer. They reported that short exposure of seeds to high temperatures generally stimulated germination whereas prolonged exposure reduced seed germination. Pretreatment of seeds of *Piliostigma thonningii* with tetraoxosulphate VI acid gave good germination at 30 minutes – 120 minutes acid-soaking periods than when the seeds were sown immediately (Ouattara and Louppe, 1992). However, the impact of breaking the dormancy with the acid on rate of successful establishment of seedlings has not been documented. Therefore, this study investigated the germination efficiency of breaking hard seed coat dormancy in *P. thonningii* with 1N H_2SO_4 and subsequent early growth biology under nitrogen fertilization.

Materials and Methods

Studies on germination and early growth biology of **Piliostigma thonningii* (Schum.) Milne-Redhead

(i) Germination study:

Fruits (pods) of *P. thonningii* were collected from mature trees in the three sites and in Ibadan to have a large pool of genotype. The seeds were then extracted and thoroughly mixed together in a bag before assessing them. Pre-germination treatments aimed at breaking the hard seed coat dormancy of the seeds were applied as follows:

Pre-germination treatments: Germination studies were conducted with the aim of removing the mechanical hindrance posed by hard seed coat using 1N H_2SO_4 (acid scarification). *P. thonningii* was selected by virtues of its importance as wildlife feed and irregularity of occurrence in the three wetlands. The acid scarification treatment of the seeds of the forage legume, *Piliostigma thonningii* was tried for 0, 2, 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 110 and 120 minutes with distilled water applied for the "0" control. 10 seeds were put in a Petri dish laid with moistened 9 cm Wattman's No 1 filter paper. The treatment were replicated thrice. The seeds were moistened regularly and number of seeds that germinated was recorded over a 14- period.

(ii) Early growth study: (Phenological Study)

Four seeds of *Piliostigma thonningii*, acid-scarified in 1N H_2SO_4 for 30 minutes and washed with distilled water were sown in thirty pots (22 cm deep with 19 cm surface diameter) which were each filled with 3.5 kg of top soil collected from the Park. They were watered regularly to avoid water stress. The seedlings were thinned to one per pot one week after emergence.

Two weeks after sowing (WAS), 50% (0.01g) and 100% (0.02g) Urea fertilizer as recommended for use in tree seedling production (Equ. 1-6) were applied to each of ten pots designated for each treatment and containing one plant per pot. A set of another ten pots were designated as control (0% or 0.00g Urea). These were only watered for the duration of the experiment (3 months) with no application of the fertilizer.

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Data were collected forthnightly on number of leaves/plant, stem diameter measured with a Venier Calliper, plant height measured with a meter rule, and leaf area/plant determined by graphical estimation, whereby the margin of each leaf was traced on a standard graph sheet, and the area covered estimated.

Physicochemiacl properties of the study sites

Samples of top soil from three purposively selected wetlands in Old Oyo National Park were obtained with a Soil auger from the top 0-15 cm. They were collected in three replicates and bulked to obtain a composite sample per site before dividing into three coded replicates for physical and chemical analyses at the Central Laboratory of the Institute of Agricultural Research and Training (I.A.R.& T.), Moor Plantation, Ibadan, Nigeria according to the official methods of analysis described by the Association of Official Analytical Chemist (1984). The parameters determined included routine macro nutrients (Total Nitrogen, Available Pphosphorus, Potassium, Calcium and Magmessium), Organic Carbon, pH, Exchangeable acidity, C.E.C.,and textural composition (using USDA classification system).

Fertilizer calculations

Application doses of Urea fertilizer, being a solely Nitogen pertilizer (to ammend Nitrogen deficiency in the soil) was based on recommended rate for low maintenance level (for newly planted trees) = 0.1 lb/sq ft Nitrogen

Where 1lb/sq ft = 450g/9 sq m………….. (Equ. 1)
0.1 lb = 45 g.
If 45 g/ 9 sq m N is recommended, amount needed for 1 hectare will be
$\frac{0.045\ Kg}{9} x \frac{x10000\ m^2}{1}$ ……………………… (Equ. 2)
= 50 Kg active Nitrogen/ha.
Available inorganic source of Nitrogen = Urea fertilizer.
Urea contains 45% N (45g N in 100g Urea.
1 ha furrow slice of soil weigh 2 x 10^6 Kg
Weight of soil per pot used = 3.5 Kg.
Amount of urea required per pot (containing 3.5 kg soil) =

$\frac{x\text{Soil weight /pot (Kg)}}{\text{2 x 106 Kg Soil}} X \frac{x\text{Recommended Rate}}{\text{Concentration of N}} X100$………………. (Equ. 3)
However, since the amount will be too small to weigh with tha available weighing Balance, it would be converted to grammes by multiplying the above equation by 1000.
Thus:
50 Kg N/ ha (100%) = $\frac{3.5\ x}{2\ x\ 10^6} \frac{50}{45}$ x $\frac{100}{1}$ x $\frac{1000}{1}$ …………….. (Equ.4)
$50\ Kg\ N/\ ha\ (100\%) = \frac{3.5}{2x10^6} x \frac{50}{45} x \frac{100}{1} x \frac{1000}{1}$ ………………. (Equ. 5)
= 0.19g Urea (N)/ pot.
For 50% rate, the equation will be
$50\ Kg\ N/\ ha\ (100\%) = \frac{3.5}{2x10^6} x \frac{25}{45} x \frac{100}{1} x \frac{1000}{1}$ ………………. (Equ. 6)
= 0.09g Urea (N)/pot.

Results

The soil analysis conducted on the soils of the study sites revealed that the soils of the three sites are almost similar. The soils of the wetlands of Ibuya and Ipade-Aya belonged to sandy loam textural class while the soil of the wetland of Ajaku was a loam (Table 1). The pH value

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of the wetland of Ipade-Aya was higher than those of the other two wetlands. Ipade-Aya had the highest available phosphorus, calcium and potassium. It had the highest Cation exchange capacity (CEC), and basic salts. But its organic carbon and nitrogen were the least of the three wetlands. The wetland of Ajaku had least pH value, sodium and available phosphorus. It had intermediate quantities of potassium, calcium, organic carbon and total nitrogen.

Table 1: Soil chemistry and particle size distribution (n=3 ±SE) in the rooting layers of plants in the wetlands of Ibuya river, Ipade-Aya and Ajaku River in Old Oyo National Park.

Parameter/Site	Ibuya	Ipade-Aya	Ajaku
pH (1N in H20)	6.383±0.090	6.637±0.080	6.19±0.070
CEC (molkg-1)	2.83±0.346	3.533±0.354	3.153±0.146
Ex_AC	0.467±0.0082	0.533±0.082	0.4±0.000
B._Sat	83.533±1.675	84.66±1.654	82.933±5.563
Org._C. (me 100g-1)	2.11±0.123	1.067±0.071	1.563±0.104
Total_N (me 100g-1)	0.45±0.128	0.220±0.014	0.303±0.047
Av._P (me 100g-1)	28.063±1.665	31.813±2.893	8.407±1.249
Ca (me 100g-1)	0.32±0.707	0.397±0.011	0.36±0.019
Mg (me 100g-1)	0.18±0.007	0.167±0.027	0.18±0.014
Na (me 100g-1)	1.523±0.29	1.333±0.182	1.33±0.129
K (me 100g-1)	0.673±0.164	1.163±0.131	0.847±0.130
Sand (%)	79.8±1.414	80.467±1.654	59.133±4.321
Silt (%)	14.067±0.817	14.06±2.160	22.067±6.377
Clay (%)	6.133±1.633	5.467±0.817	18.8±2.450
Textural Class	Loamy sand	Loamy sand	Sandy loam

Germination study– Treatment of seeds of *Piliostigma thonningii* with 1N H_2SO_4 –
First Trial

Laboratory experiments were conducted on methods of breaking suspected hard seed coat dormancy of *P. thonningii* using 1N H_2SO_4 at different soaking time regimes over a fourteen day period. There were two trials, the results of which are displayed in Figures 1 and 2 below

Germination was not recorded in the control until the fourteenth day after plating (DAP), and he germination percentage was very low (13.33%) (Figure1). Also, the percentage germination was initially very low for 2 and 5 minutes soaking times (MST). 50% germination was recorded for 2 MST at 14 DAP and 60% for 5 minutes at 14 DAP. 60% and 63.33% were recorded for 10 MST and 15 MST at 7 DAP and 10 DAP respectively, both of which were not statistically different at 10 DAP ($P<0.05$). Greater responses were progressively recorded as the soaking time increases; 76.67% for 20 MST at 14 DAP. 63.63% was recorded as early as 7 DAP for 25 MST, which also had 86.67% and 96.67% at 10 DAP and 14 DAP respectively. 46.67% germinated as early as 5 DAP for 30 MST; 96.67% of the seeds germinated at 40 MST on the 14 DAP. Remaining soaking times gave 100% germination at 14 DAP. The 110 MST produced 70.67% germination as early as 7 DAP, while 120 MST produced 50% and 93.33% germination at 5 Dap and 7 DAP respectively.

Second Trial

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In the second trial (Figure 2), it was shown that the germination percentages of the *P. thonningii* seeds to varying soaking time regimes increases as the days after plating (DAP) increases. The control showed no response to the time progression. 10% germination was recorded for 2 MST at 7 DAP, 60% at ten DAP and 70% at 14 DAP. The 5 MST gave 13.33% germination at 5 DAP, the response being as high as 93.33% at 14 DAP. The response at 10 DAP was a little lower, but 76.67% was recorded at 10 DAP. The germination percentages were very high at higher DAP for other soaking times. 100% germination was recorded as from 30 MST for 14 DAP to the 120 MST.

However, results showed that seeds subjected to more than 30 minutes soaking-time exhibited varying degrees of the scorching effect of the acid, resulting in inability of some to successfully germinate.

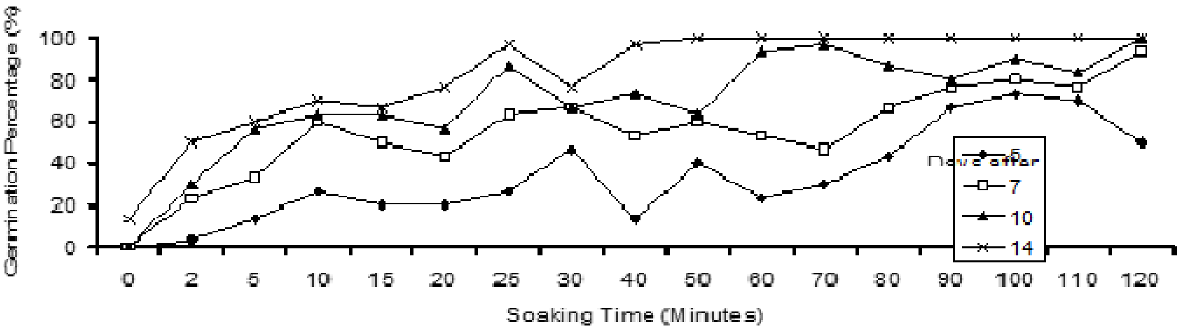

Figure 1: Germination of seeds of *Piliostigma thonninngii* at various soaking times in 1N H_2SO_4 (Second Trial)

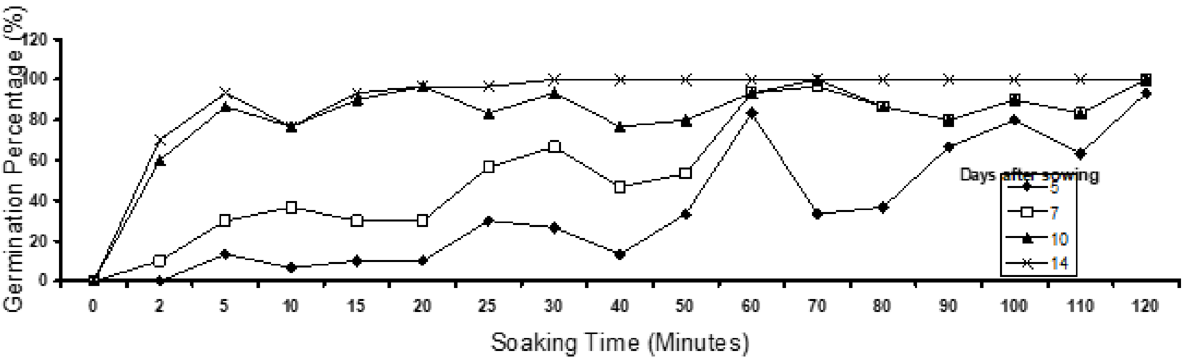

Figure 2: Germination of seeds of *Piliostigma thonninngii* at various soaking times in 1N H_2SO_4 (Second Trial)

Study of early growth biology of *Piliostigma thonningii in* nursery.

Plant Height:

The increase in height of *P. thonningii* plants under all the treatments was initially slow for the first six weeks. The height differences among the treatments were not significant ($P<0.05$). In the first six week period, plants subjected to 0% Urea fertilizer treatment gained a mean of 4.8 cm ±0.47, those under the 50% treatment gained 5.3 cm ±0.526 and the ones under 100% increased by a mean of 5.8 cm ±0.565. Over the following six weeks, mean gain in height were 12.6cm ±1.14, 14.4cm ± 1.455 and 14.1cm ±1.957 for 0%, 50% and 100% urea application (Figure 3).

Stem Diameter:

The correlation coefficient ($r=0.21$) of the Urea level against stem diameter is low and was not significant. The diameters vary from (0.21-0.30) cm for 0%, (0.21-0.31) cm for 50%, and (0.23-0.32) cm for 100% Urea applications (Figure 4). A slight rapidity in development of the diameter was noticed in the first six weeks of the study, followed by a reduction in the rate of increase (Figure 5). The differences among the treatments were not significant. Correlation analysis ($P<0.05$) accounted for a low non-significant positive relationship ($r=0.21$) between the stem diameter and the Urea levels.

Number of Leaves:

A mean number of 3, 3, and 4 leaves were produced as at 2 WAS for 0%, 50% and 100% Urea levels respectively. The number of leaves steadily increased to 12, 11 and 11 for the respective treatments at 12 WAS (Figue 5). There was hardly any difference ($P<0.05$) in the plants response to the urea treatments. This is explained by a no correlation ($r=0.00$) at $P<0.05$, which indicated a null effect of the urea fertilizer at the 50% and 100% levels.

Leaf Area

The mean leaf area of plants under each treatment increased slowly for the first 6 WAS as indicated in Figure 5: 3.43 cm^2-9.39 cm^2 (0)%, 3.65 cm^2-7.88 cm^2 (50%), and 3.23 cm^2-10.25 cm^2 (100%). The differences among the treatments were not significant. Though not significant, the rate at which control plants (0% Urea) increased in leaf area was higher than the plants on other treatments (Figure 6). Correlation analysis accounted for small non-significant negative relationship ($r= -0.09$) between the area of the plant leaves and the urea treatments.

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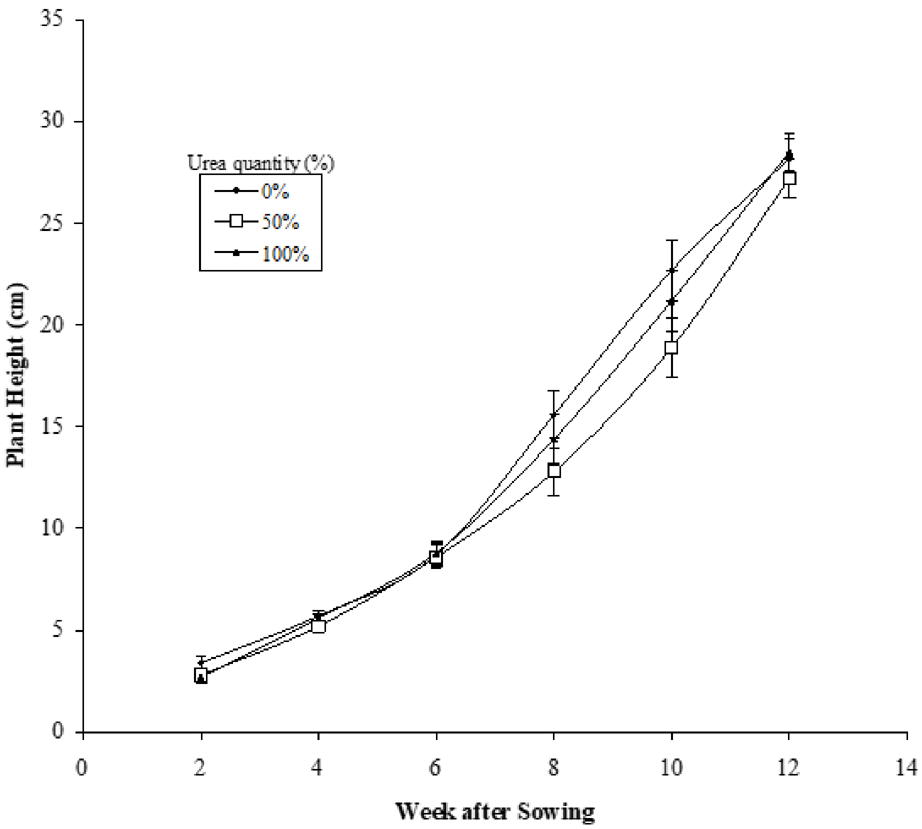

Figure 3: Response of plant height of *Piliostigma thonningii* to different rates of urea fertilizer

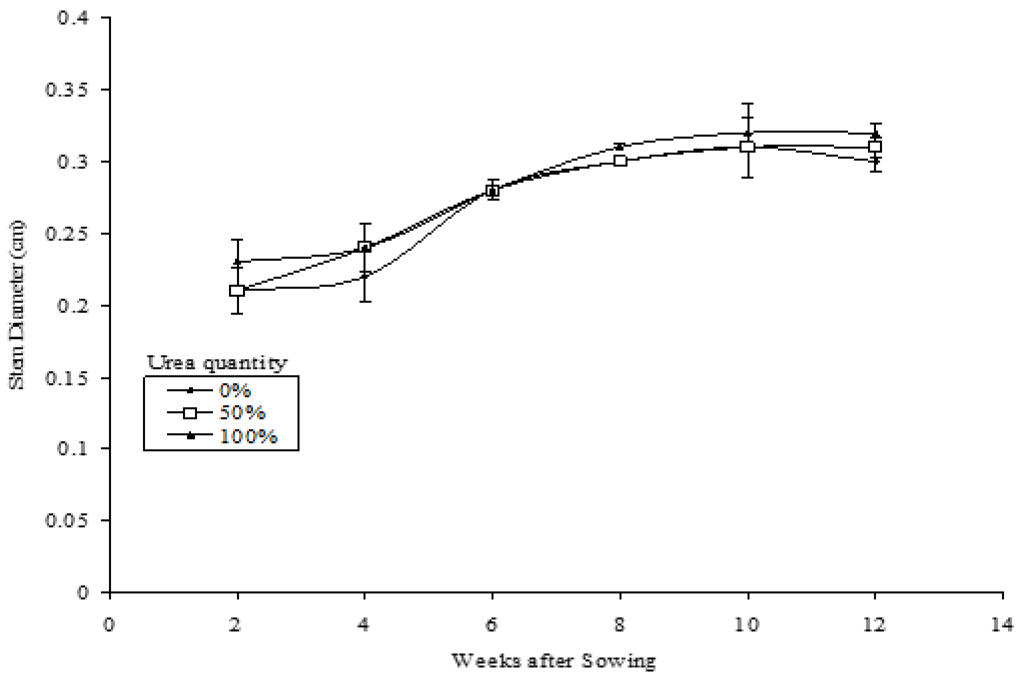

Figure 4: Response of stem diameter of *Piliostigma thonningii* to different rates of urea fertilizer

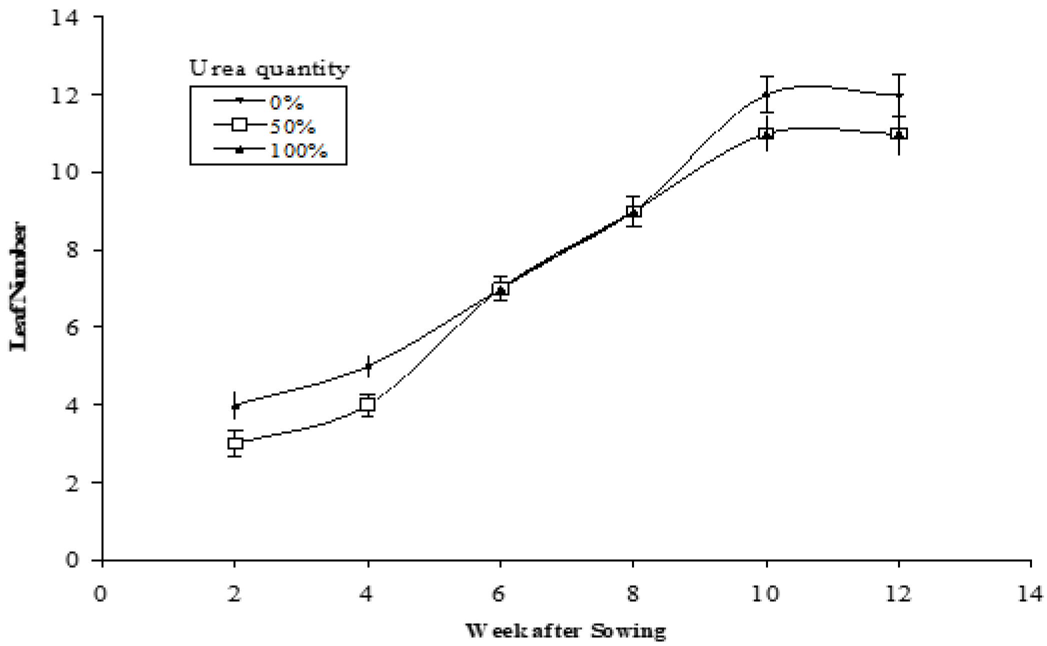

Figure 5: Response of number of leaves of *Piliostigma thonningii* to different rates of urea fertilizer

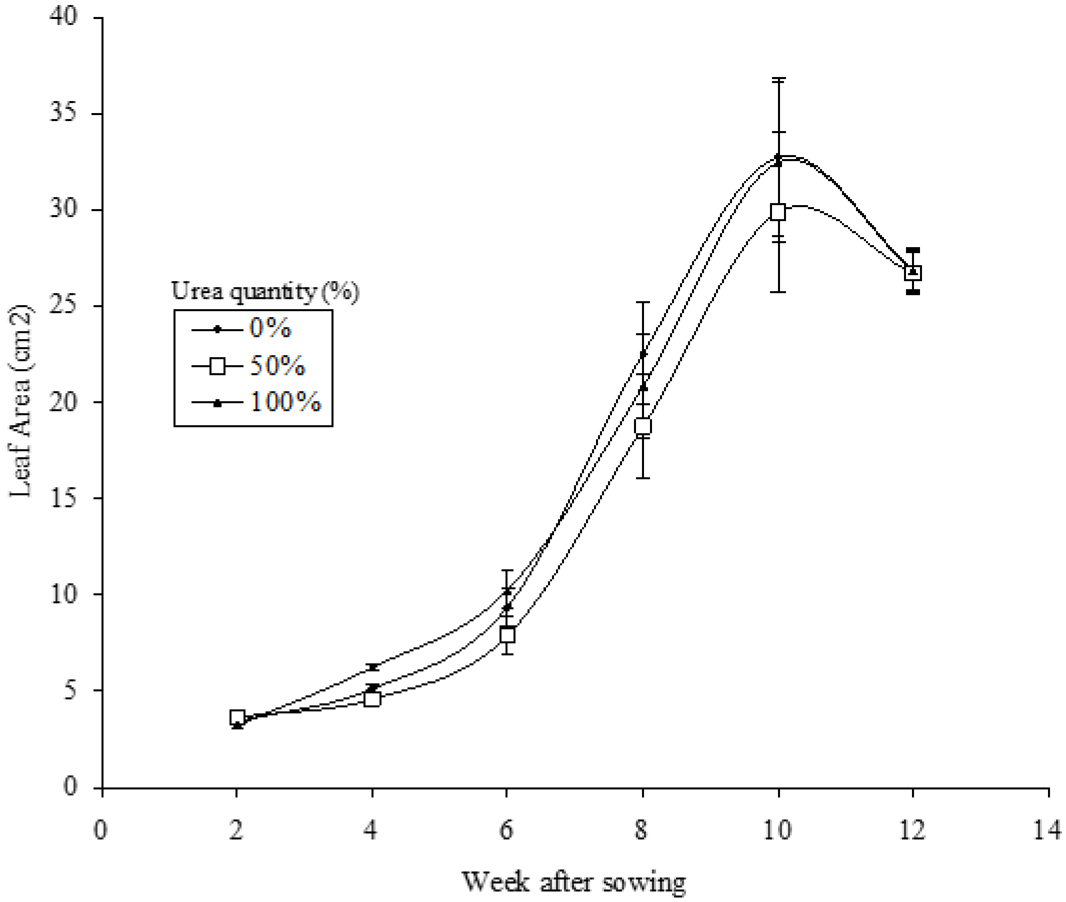

Figure 6: Response of leaf area of *Piliostigma thonningii* to different rates of urea fertilizer

Discussion and Conclusion

The population of *P. thonningii* in the wetlands could be conserved through establishment of a nursery where it could be raised until it had established enough to survive under the new fire regime before transplanting (afforestation). The nursery management would be easy to handle since the study had demonstrated that the hard seed coat dormancy could be overcome when seeds are treated for 25-30 minutes with 1N H_2SO_4. This treatment regime produced 83.33% - 93.33% germination successes at 10 days after sowing. The nursery could be used to plant up important areas where wild fauna roam in the Park.

Although, the effectiveness of breaking the seed dormancy of *Piliostigma* thonningii over a wide range of duration (30-120 minutes) as reported by Ouattara and Louppe (1992) was confirmed in this study, it was crucially discovered that treatment for lower durations gave a more uniform performance in number and growth vigour of germinated seedlings.

Piliostigma thonningii has shown through this study that it has good potentials to restore and maintain the link between wildlife conservation and availability of browse plants in Old Oyo National Park. This finding effectively corroborates the observation of Gedir *et al.* (2015) and Zeller *et al.*, (2021). Thus, it can improve the health and populations of ungulates and primates that Olubode *et al.*,(2009) reported for the Park, in the face of climate-induced degradations of vegetations in protected spaces in Nigeria, just as Raiho *et al.*, (2022) observed.

Although, nitrogen is usually reported as a limiting resource in animal communities (Keddy, 2008), the study has indicated that *Piliostigma thonningii*, being a nitrogen-fixing legume bush could ameliorate nitrogen challenge in Old Oyo National Park. The study has shown that the shrub does not need supplemental nitrogen to perform optimally at early growth stage.

Piliostigma thonningii can be easily domesticated in nursery without nitrogen fertilizer/amendment to enhance abundance and health of ungulates and primates in Old Oyo National Park. It can further serve as a climate-mitigation shrub to restore and maintain the delicate link between vegetation and fauna of the Park

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