



Effects of Watering Regime and Rhizobium Inoculation on the Growth, Functional and Yield Traits of Four Legume Species

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SOA and AJA designed the study. Authors AJA and FMO performed the statistical analysis. Authors SOA and AJA wrote the protocol, authors SOA and FMO wrote the first draft of the manuscript. Authors SOA and AJA managed the statistical analyses of the study. Author FMO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The effects of watering regime and rhizobial inoculation on the growth, nodulation and seed yields of some legume species was evaluated in the screenhouse of the Department of Crop, Soil & Pest Management, Federal University of Technology, Akure, Nigeria. The experimental layout was 5 x 3 x 2 factorial with 3 replications given a total of 90 treatments. Seeds of five staple and forage legume species: Pakala (*Phaseolus lunatus*), Soy bean (TGX 199057f and TGX198057), Stylozanthos (*Stylozanthos macrocephala*) and the Greenleaf Desmodium (*Desmodium intortum* (Mill.) Urb.) were sown into plastic pots which were watered at 4-, 8- and 12- days intervals, with or without rhizobium inoculation using a rhizobium strain, *Rhizobium phaseoli*. The plastic pots (5 litre capacity) were perforated at bottom to allow for drainage and were filled with pure river sand. The effect of watering regimes on legume species was significant on leaf development, soybean

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variety, TGX199057f had the highest number of leaves while *Desmodium* had the least number of leaves. Seedlings that were watered at 4- and 8-days interval had better vigour of growth compared to 12- days watering interval. Compared with 12-days watering interval, both inoculated and non-inoculated seedlings subjected to 4- and 8-days watering intervals had higher root and shoot biomass. TGX 199057f non- inoculated at 8-days watering intervals had the highest value for number of pods and TGX198057 watered at 12- day intervals had higher pod weight. Rhizobium inoculated legumes produced more vigorous plants in addition to higher root nodules, plant height and number of leaves compared with the non-inoculated species. The highest number of leaves and nodules were produced by *Phaseolus lunatus* when inoculated with the rhizobium strain. However, the highest number of pods was obtained for inoculated soybean (TGX 199057f) compared with the non-inoculated plants. The highest seed yield was obtained from rhizobium inoculated soybean (TGX 198057). *Desmodium* produced few seeds compared to *Phaseolus lunatus* and *Stylozanthos*. The effects of watering regimes and rhizobium inoculation varied on leaf proximate and chemical composition of legume species. The nitrogen, crude protein, ash, chlorophyll, soluble carbohydrates content in leaf differed among the legume species. The legume species inoculated and grown at 4- days watering intervals had the highest contents of moisture, nitrogen, chlorophyll, soluble carbohydrates and ash compared to non-inoculated legume species. Application of rhizobium strain significantly increased seed yield in soybean and *Desmodium* and caused substantial increase in nodulation. The study has shown that to increase legume productivity under soil moisture deficit stress of marginal/degraded soil, application of rhizobium inoculant is recommended.

Keywords: Soil moisture; stress; root nodule; legumes; growth; seed; chemical; proximate.

1. INTRODUCTION

Legumes belong to the family Fabaceae (formerly *Leguminosae*). Legumes are grown primarily for their seed, for livestock forage and silage and as soil enhancing green manure. Legumes are notable in that most of them have symbiotic nitrogen fixation –fixing bacteria in structure called root nodules [1]. Legumes are plants with seed pods that split into two halves. Edible seeds from plants in the legume family include beans, pea, lentils, soy bean and peanuts. Although legumes are an important part of traditional diets around the world, they are often neglected in typical western diet [2]. Legumes are important sources of animal protein are often rich in saturated fats, the small quantities of fats in legumes are mostly unsaturated. Legumes are among the best protein sources in the plant kingdom. Since legumes are relatively cheap compared to meat, eating more legumes may be an alternative to meat [2]. Legumes differ from grasses and cereals and other non- legume crops because much of the nitrogen they require is produced through fixation of atmospheric nitrogen by bacteria in nodules on their roots [3,4]. Legumes are economically important in addition to its use in many cropping systems because of their ability to assimilate atmospheric nitrogen by root nodules bacteria [5]. Forage legumes are adapted to acid and low fertility soils, even

though they show high responses to fertilizers. *Stylozanthos* and *Desmodium* spp. are perennial tropical forage legumes. Leguminous forage plants, characterized by their capacity to biologically fix atmospheric nitrogen in the soil, their introduction into pasture or rangelands will improve livestock diet and reducing nitrogen fertilizers, provide feed resource relevant to attain increases in animal production [4].

Legumes have the ability to form a mutually beneficial (symbiotic) relationship with certain soil bacteria of their type [5]. These bacteria can fix nitrogen from the air and make it available to the plant via symbiotic nitrogen fixation [6]. The amount of nitrogen fixed can meet the needs of the plant and leave nitrogen in the soil for the following crop. Many legumes contain symbiotic bacteria called rhizobia within root nodules of their root systems. These bacteria have their special ability of fixing nitrogen from atmospheric molecular nitrogen (N_2) into ammonia (NH_3) [7]. Under nitrogen limiting conditions, the leguminous plant will form root nodules where the bacteria are hosted and will find the proper conditions to reduce atmospheric nitrogen into ammonia [7,8]. Symbiotic nitrogen fixation largely contributes to the nitrogen nutrition of the host plant suppressing the needs for nitrogen fertilizers. Incorporating legume crops into cropping system will improve nitrogen and carbon sources to the soil for subsequent crops

[8]. Rhizobium is a common soil bacterium, not toxic to humans, plants or animals. It is one of the most beneficial bacteria to agriculture [8,9]. Rhizobia are very minor components of the soil micro flora and reach their maximum numbers in association with plant roots [6,10]. They are stimulated by carbon compounds in plant roots and are controlled by microbial competition. Their ability to infect legume roots and multiply within the resulting root nodules protected from the soil environments provides a special advantage over their competitors [11,12].

Drought considerably reduces legumes species productivity, especially in areas where agricultural system is dependent on rainfall. Drought is among the most difficult challenges faced by resource poor farmers [4]. Water stress and excess water can have adverse effects on nodulation and nitrogen fixation [13,14]. Water stress is one of the major causes of reduced growth, development and yield in leguminous plants and also reduces nitrogen fixation and its related traits [14]. Legumes are very sensitive to drought which leads to reduced yield and seed quality. Water stress is a major factor affecting symbiosis and leads to decreased nodule formulation, reduced nodule size and N₂ fixation [15,16]. Leguminous plants in association with rhizobium species have the potential to fix large amount of atmospheric nitrogen which contributes to the soil nitrogen pool provided that the nitrogen fixation is not restricted by other environmental or microbial factors [17]. Nitrogen fixation is the process whereby legume crop and specific rhizobium bacteria work together to make nitrogen from the soil air surrounding the roots available for use by the plant [14]. The N fixation process requires a considerable amount of energy which is provided by the plant. The plant provides nutrients and water to the rhizobia in the nodules and in return, the rhizobia provided fixed nitrogen to the plant. The amount of N fixed varies with the type of crop, crop health, the supply of nitrogen already available in the soil and other environmental conditions [2,18]. Rhizobium inocula are living culture of bacteria but are mostly sensitive to heat desiccation and light [19-21]. Nitrogen fixation in legumes depends on the formulation of nodules by rhizobium. Rhizobium is a common soil bacterium and among rhizobium strains commonly used as inoculants for introduction to soils are *Rhizobium phaseoli*, *R. leguminosarum* *bv trifolii* and *R japonicum*. Some rhizobium are specific and nodulated several legumes [22]. Inoculation is the process of introducing the

appropriate rhizobium bacteria to the soil in numbers sufficient to ensure successful nodulation [23].

Global legume production increased from 150 million tons in the 1980's to 300 million tons in the 2000's. Legume production is dominated by soybean while pulses accounted for approximately 20% of total production during the same period [24]. This challenge is further compounded by the severe competition for land and water from industry and urban development. Such competition pushes agriculture to marginal areas, where water-limiting conditions often constrain crop productivity. Besides the persistent water limitation and year to year fluctuations of meteorological conditions in these marginal areas (e.g., semi-arid environments) tend to be large, and these variations significantly affect food security in these rain-fed systems. Drought can have negatively impact on the yield of most cultivated crops, from monocotyledons C₄ (e.g., maize) to di-cotyledons C₃ cereals (e.g., wheat) and legumes [4,14]. The yield of food legumes are usually variable or low due to terminal drought that characterize these areas [25]. Even in non-dry land where precipitation is generally sufficient for legume (i.e., soybean) cultivation, water deficiency may still occur over a period of a few weeks, causing significant yield loss [26].

A more effective and cheaper way of raising the N status of the soil is to exploit the ability of legumes to fix appreciable quantities of atmospheric nitrogen. Therefore, the specific objectives of the study are to examine the effect of watering regime and rhizobia inoculation on the growth, seed yield and yield components of some legumes species, nodulation and nitrogen fixation capacities of some legumes species and the leaf chlorophyll and soluble carbohydrate concentration as drought tolerance traits in legumes species.

2. MATERIALS AND METHODS

The experiment was conducted in the screen house of the Department of Crop, Soil and Pest Management, Federal University of Technology Akure, located in the rain forest area of south western Nigeria from January – August 2015. In the screen house, temperature and relative humidity fluctuated between 25-31°C and 58-85°C respectively, during the period of the experiment.

2.1 Planting Materials

Seeds of five staple and forage legume species were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The species are: Pakala (*Phaseolus lunatus*), Soybean (TGX 199057f) and TGX198057, Stylo (*Styloanthus macrocephala*) and the Greenleaf Desmodium (*Desmodium intortum* (Mill.) Urb.). The seeds were planted into 5 liter buckets perforated at bottom to allow for drainage. The pots were filled with pure river sand obtained from a flowing river in Akure. A rhizobium strain, *Rhizobium phaseoli*, was obtained from the International Institute of Tropical Agriculture, Ibadan.

2.2 Treatments and Experimental Design

The experimental layout was 5 x 3 x 2 factorial arranged in a Completely Randomized Designed (CRD) with 3 replications (total of 90 treatments). Treatments consisted of 5 staple and forage species, 4, 8 and 12 days watering intervals and with or without rhizobium inoculation. At each watering interval, 900 ml of water was applied throughout the experiment.

2.3 Data Collection

Measurement of agronomic variables commenced 3 weeks after planting and lasted for a period of 8 months. The variables include number of leaves, plant height, number of branches, number of nodules, nodules weight, nodules diameter, number of pods, pod weight, number of seeds, seeds weight, days to 50% flowering, soil chemical properties. Leaf samples were collected and analyzed for soluble carbohydrate, chlorophyll, ash, nitrogen, crude protein, moisture content and crude fibre. These parameters were determined in the following ways: The number of leaves per plant: This was estimated by counting. The plant height: This was measured using measuring tape. The measurement was taken from the base to the apical shoot. The number of branches per plant: This was estimated by counting. Number of nodules: This was done by visual counting of nodules borne on root after terminating the experiment. Nodule weight: The nodules of each plant were removed and weighed using a weighing balance. Nodule diameter: This was measured with the use of Vernier calliper. Number of pods: This was done by visual counting of pods borne on each plant at harvest. Number of seeds: This was done by visual

counting of seeds after removal from pod. Seed weight: This was done by weighing the seeds on weighing balance. The date to 50% flowering: This was determined when the plants produce maximum flowers during the growth stage.

2.4 Determination of Selected Soil Physical and Chemical Properties

The soil texture was determined using part of the soil samples collected for the experiment; this was set aside before the contamination of the remaining part. The soil was air-dried to reduce the moisture content after which it was taken to the laboratory where the soil texture class was determined as sandy-loam as defined according to FAO/USDA soil classification system. Soil pH was determined by using 1:1 water suspension by adding 10 ml distilled water to 10 g of soil. The suspension was stirred continuously for 30 minutes allowed to stand for 20 minutes. After calibrating the pH meter with buffer solutions of pH 4.0 and 7.0, the pH was read by immersing the electrode into the upper part of the suspension. Organic carbon was determined by a modified Walkley-Black wet oxidation method. Soil organic matter of the soil sample was calculated by multiplying the per cent organic carbon by a Van Bemmelen factor of 1.724. Potassium and sodium in the soil extract were determined by flame photometry. Standard solutions of 0, 2, 4, 6, 8 and 10 ppm K and Na were prepared by diluting appropriate volumes of 100 ppm K and Na solution to 100 ml in volumetric flask using distilled water. Flame photometer readings for the standard solutions were determined and a standard curve constructed. Potassium and sodium concentrations in the soil extract were read from the standard curve. Available P was determined using the Bray P1 method. The method is based on the production of a blue complex of molybdate and orthophosphate in an acid solution. A standard curve was constructed using the readings. The concentration of P in the extract was obtained by comparison of the results with a standard curve. For the determination of calcium, a 10 ml portion of the extract was transferred into an Erlenmeyer flask. To this, 10 ml of potassium hydroxide solution was added followed by 1 ml of triethanolamine. Few drops of potassium cyanide solution and few crystals of cal-red indicator were then added. The mixture was titrated with 0.01 M EDTA (ethylene diaminetetra acetic acid) solution from a red to a blue end point. The exchangeable magnesium alone was calculated

by subtracting the value obtained from calcium alone from the calcium + magnesium value.

2.5 Extraction and Determination of Leaf Chlorophyll

Chlorophyll extraction and its determination were done at the laboratory of the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure. The 2 uppermost leaves of legumes species from each treatment were harvested. One gram of the fresh plant samples were cut into pieces and smashed in a mortar. The samples were put in a test tube and its chlorophyll content was repeatedly extracted with successive volume of 100 ml acetone/water (80:20 v/v) until no traces of green colour were noticed (residue became white). While adding the solvent (acetone), the test tubes containing the samples were kept boiling in hot water bath. The total volume of the extract was also recorded at end of the extraction. Three millimeter (3 ml) of the extract was taken and the absorbance was determined with a spectrophotometer (Spectronic 20) at two wave length of 663 and 645 nm that corresponds to maximum absorption of chlorophyll "a" and "b" respectively. The total chlorophyll content was calculated as follows:

$$\text{Total chlorophyll content (mg/100 g tissue)} = (20.2A_{645} + 8.02A_{663}) (V/10 w)$$

Where, A_{645} = absorbance at 645 nm wavelength; A_{663} = absorbance at 663 nm wavelength,

V = final volume (cm^3) of chlorophyll extract in 80% acetone and W = fresh weight (g) of tissue extracted

2.6 Determination of Leaf Water Soluble Carbohydrate

About 2 ml of extracts were pipetted into a test tube. 10 ml of anthrone reagent was rapidly added and mixed by shaking and placed in a boiling water bath. The absorbance of the extract was determined on a spectrophotometer device (using a 10 mm diameter cuvette). About 0.5 g of plant samples were ground and transferred into 250 ml test tube and 220 ml of water was added. The bottles was capped and shaken on a shaker for about an hour and filtered. The first few ml was ejected and the filtrate was retained for the determination of soluble carbohydrate using Anthrone reagents. 770 ml of concentrated H_2SO_4

was added to 330 ml of distilled water, in addition to 1 g of thiourea, 1 g of antrone, stirred until dissolved and was stored in a refrigerator. Glucose stock solution, 1 g of anhydrous D (+) glucose in water and diluted to one litre prepared immediately before use. From the glucose working standard solutions, 10 ml of stock to 100 ml was diluted to produce 100 ppm. From these, 0, 5, 10, 20, 40, 80ml was pipetted and made up to 100 ml and these produced 0, 5, 10, 20, 40, 80 ppm. Samples of 2 ml of each glucose working standard solutions were pipetted into the glass test tube and rapidly, 10 ml of anthrone reagent was added and mixed by shaking. The test tube was loosely covered with a glass bulb stopper and placed immediately in boiling water for 20 minutes. The absorbance was measured using spectrophotometer device in a 10 mm optical cell at 620 nm. The graph of absorbance was plotted against glucose concentration in ppm and prepare standard graph with each batch of extracts examined. The glucose standard becomes 0, 0.8, 1.7, 3.3, 6.7, 13.3 ppm respectively.

Data collected on plant and soil parameters were subjected to analysis of variance (ANOVA) and treatment means were separated using Least Significant Difference (LSD) at 5 % level of probability and Duncan Multiple Range Test (DMRT).

3. RESULTS

3.1 Pre-experiment Soil Chemical Properties

Table 1 shows the result of soil chemical properties before the experiment. The pH of the soil was 5.11. Organic matter contents analysed was 1.11% Nitrogen content was 0.07 g/kg. The available P content in the soil was 0.73 mg/kg, K (0.14 cmol/kg), Na (0.24 cmol/kg), Ca (1.89 cmol/kg), Mg (1.29 cmol/kg).

Table 1. Pre-experiment soil chemical properties

Chemical properties	Values
pH (H_2O)	5.11
SOM (%)	1.11
Total N (%)	0.05
Available P (Bray-1) (mg/kg)	0.73
Exch. K (cmol/kg)	0.14
Exch. Na (cmol/kg)	0.24
Exch. Ca (cmol/kg)	1.89
Exch. Mg (cmol/kg)	1.29

3.2 Effects of Rhizobium Inoculation on Soil Chemical Properties

Table 2 showed the result of soil chemical properties at the end of the experiment. The inoculated legume species had higher pH. *Desmodium* and TGX 198057 had higher chemical properties than other legume species. There was no significant difference ($P < 0.05$) for Nitrogen. The same trend was observed for Na, Ca and available P. The increase in the nutrients content was significantly higher than pre – experiment status ($P > 0.05$).

3.3 Effects Watering Regimes on Growth of Legume Seedlings

There were significant differences ($p < 0.05$) in the responses of legume seedlings on the measured growth parameters. The time course of the effects of species on plant height is presented in Table 3. *Phaseolus lunatus* watered 4-days was the tallest while TGX 199057f had the lowest height at 12 days watering interval compared with *Stylozanthos* and *Desmodium* which had the highest plant height at 8 days watering interval. Significant differences occurred between species for number of branches. legume seedlings watered at 4- days interval had the highest number of branches compared with 8 and 12 days watering interval. Similarly, the effect of watering regimes on legume species was significant on number of leaves except at 3 weeks after planting (Table 3) there seems to be no consistent trend in leaf production in *Phaseolus lunatus* at 4 days watering interval. Significantly TGX199057f had the highest number of leaves across the period of measurement while *Desmodium* had the least number of leaves.

3.4 Effects of Watering Regimes on Growth and Yield Parameters of Legume Species

Table 4 shows the effects of watering regime on growth character of legume seedlings. Seedlings

that were watered at 4 and 8 days interval enhanced high growth character compared to 12 days watering intervals across the different periods of observation. Table 6 shows the effects of watering regimes on growth and yield parameters of legumes seedlings. The seedlings that were watered at 4 and 12 days interval had higher number of nodules, pods, seed weight, flowers and longer root compared to 12 days watering interval.

3.5 Effects of Rhizobium Inoculation on Growth Parameters of Legume Seedlings

Rhizobium inoculated legume seedlings produced significantly higher growth characters especially the plant height, number of leaves compared with the non-inoculated species. (Table 5). Plant height was significantly affected by treatments (Table 5). Highest plant height was observed in inoculated *Phaseolus* compared with the non- inoculated. The mean values for legumes species also show significant difference in plant height. Rhizobium inoculation treatments also gave superior results compared with the non-inoculated. Rhizobium inoculation enhanced number of leaves (Table 5). More leaves were found in inoculated plant compared with non-inoculated. Among the legume species differences in values indicates that the five legume species behaved differently, however highest number of leaves were produced by inoculated *Phaseolus*.

3.6 Effects of Rhizobium Inoculation on Growth and Yield Parameters of Legume Species

Table 6 shows the effects of rhizobium inoculation on number of roots per plant, number of nodules per plant, number of pods and seed yield per plant. The number of roots per plant was significantly affected by various treatments. Higher number of roots was produced by *Phaseolus lunatus* inoculated with rhizobia.

Table 2. Soil chemical properties taken from the legume species at termination of experiment

	pH (1:2 H ₂ O)	Om (%)	N (%)	P (cmol/kg)	K (cmol/kg)	Na (cmol/kg)	Ca (cmol/kg)	Mg (cmol/kg)
<i>Phaseolus lunatus</i>	6.76c	1.63b	0.56a	1.63b	0.61bc	0.46bc	1.53c	0.77b
TGX 199057f	6.82b	1.29c	0.52a	1.29c	0.70a	0.50b	1.41d	0.79b
TGX 198057	6.97a	1.72b	0.49a	1.72b	0.65ab	0.48bc	1.66a	1.03a
<i>Stylozanthos</i>	6.77c	1.38a	0.54a	3.38a	0.56c	1.94a	1.69a	0.84b
<i>Desmodium</i>	6.94a	1.76b	0.54a	1.76b	0.64ab	0.45c	1.60b	0.81b

Means along the column bearing same letter are not significantly different by DMRT ($p=0.05$)

Rhizobial inoculation had significant effect on number of nodules per plant (Table 6). Higher number of nodules was produced by *Phaseolus* inoculation. The Treatments imposed had significant effect on number of pods per plant (Table 6). However, the highest number of pods per plant was observed in inoculated soy bean (TGX 199057f) compared with the non-inoculated plants. The highest seed yield was obtained from soy beans (TGX 198057) that were inoculated. *Desmodium* produced few seeds compared to *Phaseolus* and *Stylozanthus* which did not produce any seed (Table 6).

3.7 Interaction Effects of Legume Species, Rhizobium Inoculation and Watering Regime on Growth and Yield Parameters

Significant interactions were obtained on growth and yield of legume species between rhizobial inoculated species and watering regime for root weight and root length. Table 7 shows that both inoculated and non-inoculated seedlings subjected to 4- and 8 -days watering intervals were consistently higher compared to inoculated and non-inoculated seedlings subjected to 12- days watering interval. However, non- inoculated *Phaseolus lunatus* at 4-days watering interval had highest number of nodules while TGX 199057f non- inoculated at 8-days watering intervals had the highest value for number of pods and TGX198057 at 12 days had higher pod weight. The interaction effects of rhizobium inoculation and watering regime for plant height of legume species were significant. Species inoculated and watered at 4 and 8-days intervals were taller than those watered at 12days intervals. Consistently higher number of leaves were obtained for both the inoculated and non-inoculated at 4- and 12-day intervals while plants watered at 12- days interval had lower number of leaves except for *Stylozanthus* that had high number of leaves in all watering regimes Table 9.

3.8 Interaction Effects of Watering Regimes and Rhizobium Inoculation on Leaf Proximate and Chemical Composition of Legume Species

The nitrogen, crude protein, ash, chlorophyll, soluble carbohydrates content in leaf differed among the legume species. However, legumes species inoculated and grown at 4- days watering intervals had the highest nitrogen content chlorophyll, soluble carbohydrates, ash,

and moisture content compared to non-inoculated legume species (Table 8). There were significant differences among the legume species for leaf proximate and chemical composition (Table 9). All the treatment gave higher effective chemical properties except for the chlorophyll content which was low in all the legume species.

4. DISCUSSION

4.1 Effects of Watering Regime on Growth and Development of Legume Seedlings

The results of this study showed that the measured growth variables of legume species responded to the watering regimes imposed. The enhancement of plant growth by more frequent watering may be attributed to higher moisture contents in the crop root zone. Hamidou et al. [27] stated that, water stress reduces plant growth through inhibition of various physiological and biochemical processes, such as photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism, and hormones.

4.2 Effects of Legume Species on Growth and Development as Affected by Watering Regime and Rhizobium Inoculation

The measured growth variables of inoculated legume seedlings were statistically superior under 4- and 8 - days watering intervals compared with 12- days. This implies that the legume seedlings require consistently moist root zone environment and favourable microclimate. [28,29]. Legumes are known to be very sensitive to a soil water deficit [30, 31]. Adequacy of soil moisture promotes leaf development as was obtained for seedlings that were watered 4- day possibly via enhance evapotranspiration. Adequate soil and plant water status is critical to the survival of seedlings during establishment. The results of this study confirmed that legume seedlings cannot withstand soil moisture deficit stress as was obtained for seedlings that were watered at 12- days intervals [31]. The more stressful situation of 12- days watering interval was characterized by high intensity of soil and air moisture deficits, these conditions have implications for survival and establishment of seedlings. Plants exposed to soil water deficit, exhibited number of physiological responses in an effort to conserve water. These include closing of stomata and arresting cellular growth

[32]. If water stress is not alleviated, plants will close stomata and shut down photosynthesis, carbon assimilation, and normal metabolism [31]. These responses mean that plants experiencing water stress will end up smaller and poor in vigour [33]). Among the legume species, drought stress reduces leaf area, number of leaves and branches [34]. Rhizobium inoculation of legume seedlings significantly increased vigour of growth. Species inoculated with rhizobium recorded maximum root and shoot biomass compared to non-inoculated.

4.3 Interaction Effects of Watering Regime and Rhizobium Inoculation on Plant Growth

Legume seedlings inoculated with Rhizobium and subjected to different 4 day watering intervals were more vigorous going by the measured growth parameters. Nevertheless, there were no significant differences for most of the measured parameters between weeks 8 and 12 and for Rhizobium inoculated seedlings. Across the species, soya bean seedling species

that were subjected to 4- day watering interval and Rhizobium inoculation have significantly ($p < 0.05$) higher growth than 8 and 12 day watering regime. These findings support the observations of Levy and Rirkum [35] who reported that rhizobium inoculation increased soil water extraction and root hydraulic conductivity. Ali [23] reported that leaf development is a sensitive parameter to drought stress. In some legumes, mild water stress and rhizobium inoculation enhanced growth than the non-inoculated [36,37]. In conformity with the findings of the present study, Osunubi and Mulong [38] reported that rhizobium inoculation enhanced leaf number in *Acacia species* (*A. albida* and *A. nilotica*). The interaction effects between rhizobium inoculation and watering regimes was not significant on the measured (plant height, number of leaves and branches) of soya bean seedlings. Some of the measured growth parameters of soya bean seedlings in this study were promoted by rhizobium inoculation. For other parameters, the non-inoculated plants exhibited similar growth responses as the inoculated.

Table 3. Effect of rhizobium inoculation on growth parameters of Legume species

Legume species	Rhizobium inoculation	Plant height (cm)	Number of branches	Number of leaves
<i>Phaseolus lunatus</i>	Inoculated	67.60c	6.42b	35.95b
	Non – inoculated	39.46a	12.58c	25.50b
TGX 199057f	Inoculated	38.48a	14.42b	29.33a
	Non- inoculated	32.12b	15.25a	24.33b
TGX 198057	Inoculated	47.05a	14.92a	31.67a
	Non - inoculated	46.56b	14.25a	28.33b
<i>Stylozanthos</i>	Inoculated	40.20c	15.33a	29.00a
	Non – inoculated	80.81a	27.42a	26.50a
<i>Desmodium</i>	Inoculated	72.59b	21.75b	26.50a
	Non – inoculated	71.02b	16.83c	26.17a

Means along the column for each legume specie bearing same letter are not significantly different by DMRT ($P = 0.05$)

Table 4. Effect of watering regimes on growth characters of legume species

Legume species	Watering regimes	Number of leaves	No of branches	Plant height (cm)
<i>Phaseolus lunatus</i>	4-day	41.30a	4.79c	28.03b
	8-days	34.94a	4.56c	22.68b
	12-days	29.49a	5.23c	22.81b
TGX 199057f	4-days	24.60a	7.75b	28.21a
	8-days	23.44a	7.50b	28.17a
	12-days	19.52a	8.25b	23.96ab
TGX 198057	4-days	29.80a	13.79b	32.21a
	8-days	31.08a	12.35b	23.04a
	12-days	25.46a	11.71b	25.25a
<i>Stylozanthos</i>	4-days	54.63a	15.81c	27.61b
	8-days	51.35a	14.94b	21.33c
	12-days	54.75a	11.94b	19.58c
<i>Desmodium</i>	4-days	36.34a	12.69c	23.31b
	8-days	38.24a	10.31c	21.96b
	12-days	34.39a	10.25c	18.77b

Means along the column for each legume specie bearing same letter are not significantly different by DMRT ($P = 0.05$)

Table 5. Effect rhizobium inoculation on growth and yield parameters of legume species

Legumes species	Rhizobium	Total nodule weight (g)	No of nodules	Nodule diameter (mm)	No of pods	Pods weight	No of seeds	Seeds weight (g)	Root weight (g)	Roots length (cm)	Days to 550% flowering	No of flowers
<i>Phaseolus lunatus</i>	Inoculated	6.21	34.42	3.76	0	0	0	0	23.09	34.42	0	0
TGX 199057f		0.65	2.27	0.85	16.8	6.69	28.71	4.24	2.75	30.5	18.92	15.82
TGX 198057		0.57	1.13	2.24	18.11	8.48	40.45	7.18	1.96	31.81	28.38	17.94
<i>Stylozanthos</i>		0	0	0	0	0	0	0	5.06	20.06	0	4.89
<i>Desmodium</i>		0	0	0	0	0	224.44	1.63	27.24	38.66	61.66	17.62
	LSD	0.32	0.54	0.23	1.1	0.32	6.12	0.12	1.1	4.6	4.21	2.12
<i>Phaseolus lunatus</i>	Non-inoculated	3.43	8.26	2.48	0	0	0	0	19.08	36.7	0	0
TGX 199057f (Soya bean)		0	0.32	0.07	23.49	7.01	36.54	4.73	0.5	22.84	19.9	15.33
TGX 198057(soya bean)		0.16	0.32	0.67	21.53	3.79	28.06	5.87	2.25	34.25	29.36	13.21
<i>Stylozanthos</i>		0	0	0	0	0	0	0	5.22	15.33	0	1.96
<i>Desmodium</i>		0	0	0	0	0	112.71	0.98	13.54	35.4	67.85	15.17
	LSD	4.49	1.3	0.73	2.53	0	20.14	4.48	3.12	8.89	9.08	1.47

Table 6. Effect of watering regimes on growth and yield parameters of legume species

Legumes species	Watering regimes	Root weight (g)	Root length (cm)	Pod weight (g)	No of pods	Seeds weight (g)	No of seeds	Days to 50% flowering	No of flowers	Total nodule weight(g)	Nodule diameter (cm)	No of nodules
<i>Phaseolus lunatus</i>	4-days	31.37a	40.13a	0	0	0	0	0	0	7.35a	3.77a	16.28a
	8-days	12.56b	34.25a	0	0	0	0	0	0	4.89b	3.33a	9.23b
	12-days	19.33c	32.3a	0	0	0	0	0	0	2.2c	2.27b	7.78b
TGX 199057f (Soya bean)	4-days	0.98a	34.25a	6.85a	18.59a	4.89a	38.17a	0	14.68a	0	0.59a	0.97a
	8-days	0.99a	28.14b	6.85a	19.33b	4.16b	30.34b	28.38b	16.88b	0.98a	0.73b	2.43b
	12-days	2.91c	17.62c	6.85a	22.51c	4.4c	29.36c	29.85b	15.17c	0	0.05c	0.49c
TGX 198057 (Soya bean)	4-days	0.98a	34.25a	6.85a	18.59a	4.89a	38.17a	0	14.68a	0	0.59a	0.97a
	8-days	1.27b	40.86b	9.54b	20.8b	5.38b	34.74b	28.38b	16.15b	0	0	0
	12-days	1.22b	24.96c	8.81c	21.04b	6.36c	36.21b	28.38b	21.04b	0.37	1.22b	0.73b
<i>Stylozanthos</i>	4-days	7.34a	19.08a	0	0	0	0	0	0	0	0	0
	8-days	3.67b	19.82ac	0	0	0	0	0	0	0	0	0
	12-days	4.4c	14.19a	0	0	0	0	0	0	0	0	0
<i>Desmodium</i>	4-days	25.2a	29.85a	0	0	1.1a	172.73a	64.1a	0	0	0	0
	8-days	17.62b	40.13b	0	0	1.35b	181.3b	64.1a	0	0	0	0
	12-days	18.35c	41.1b	0	0	1.47c	151.69c	66.06c	0	0	0	0

Means along the column bearing the same letter are not significantly different by DMRT (P = 0.05)

Table 7. Interaction effects of rhizobium inoculation and watering regimes on growth and yield parameter of legume species

Legume species	Rhizobium inoculation	Watering Regimes	No of nodules	Total no nodules (g)	Root length (cm)	Pod weight (g)	No of pods	No of leaves	No of seeds	Seed weight (g)	Root weight (g)	Days to 50% flowering	
<i>Phaseolus lunatus</i>	Inoculated	4 –days	12.15	6.39	29.36	0	0	48.27	0	0	34.35	0	
		8-days	18.47	9.79	34.25	0	0	42.28	0	0	16.8	0	
		12-days	11.18	2.45	39.64	0	0	37.14	0	0	18.11	0	
	Non inoculated	4-days	20.41	8.32	50.89	0	0	34.32	0	0	28.38	0	
		8-days	0	0	34.25	0	0	27.6	0	0	8.32	0	
		12 –days	4.37	1.96	24.96	0	0	21.85	0	0	20.55	0	
		LSD		1.41	1.94	0.53			10.4				1.94
	TGX 199057f	Inoculated	4-days	0.97	0	48.44	9.3	16.64	27.6	43.06	5.38	1.47	0
			8-days	4.86	1.96	23.49	4.4	15.66	25.76	22.51	3.43	1.47	28.38
			12-days	0.97	0	19.57	6.36	18.11	22.34	20.55	3.92	5.32	28.38
Non inoculated		4-days	0.97	0	20.06	4.4	20.55	21.6	33.28	4.4	0.49	0	
		8-days	0	0	32.79	9.3	23	21.11	38.17	4.89	0.51	28.38	
		12-days	0	0	15.66	7.34	26.91	16.71	38.17	4.89	0.49	31.32	
	LSD		0.44	1.1	0.66	1.1	0.66	13.5	1.1	0.44	0.66	1.1	
TGX 198057	Inoculated	4-days	1.94	0.98	36.7	9.79	17.13	31.76	56.76	10.77	2.94	28.38	
		8-days	0	0	34.25	8.81	19.08	34.08	33.28	5.38	1.47	28.38	
		12-days	1.46	0.73	24.47	6.85	18.11	27.11	31.32	5.38	1.47	28.38	
	Non inoculated	4-days	0.97	0.49	29.85	8.32	18.11	27.84	6.85	4.89	4.7	31.32	
		8-days	0	0	47.47	10.28	22.51	28.09	36.21	5.38	1.08	28.38	
		12-days	0	0	25.45	10.77	23.98	23.8	41.1	7.34	0.98	28.38	
	LSD		0.68	0.79	0.1	0.78	0.1	15.4	0.78	0.68	0.1	0.78	
<i>Stylozanthes</i>	Inoculated	4-days	0	0	22.02	0	0	68.09	0	0	4.89	0	
		8-days	0	0	23.49	0	0	63.37	0	0	4.89	0	
		12-days	0	0	14.68	0	0	72.37	0	0	5.38	0	
	Non inoculated	4 –days	0	0	16.15	0	0	41.17	0	0	9.79	0	
		8-days	0	0	16.15	0	0	39.34	0	0	2.45	0	
		12-days	0	0	13.7	0	0	37.14	0	0	3.43	0	
	LSD		-	-	0.14	-	-	17.2	-	-	0.14	-	
<i>Desmodium</i>	Inoculated	4-days	0	0	34.25	0	0	42.15	222.65	1.47	35.23	59.7	
		8-days	0	0	40.62	0	0	40.56	247.6	1.47	23.98	61.66	
		12-days	0	0	41.1	0	0	34.32	203.07	1.96	22.51	63.61	
	Non inoculated	4-days	0	0	25.45	0	0	30.53	122.82	0.73	15.17	68.51	
		8-days	0	0	39.64	0	0	35.92	114.99	1.22	11.26	66.55	
		12-days	0	0	41.1	0	0	34.45	100.31	0.98	14.19	68.51	
	LSD				0.68		9.4	1.46	0.78	0.68	1.46		

Table 8. Interaction effect of watering regime and rhizobium inoculation on leaf proximate chemical composition of legumes species

Legume species	Inoculation or not	Watering Regimes	Soluble carbohydrate (mg/kg)	Moisture content (%)	Chlorophyll (mg/kg)	Ash (%)	Crude fibre (%)	Crude protein (%)	Nitrogen (%)	
<i>Phaseolus lunatus</i>	Inoculated	4 - days	12.52	63.98	0.06	3.09	5.70	35.64	5.71	
		8 - days	12.48	64.88	0.12	4.27	5.36	33.51	5.36	
		12 - days	11.2	59.42	0.07	1.33	5.08	31.78	5.18	
	Non –inoculated	4 - days	11.92	50.26	0.03	2.99	4.73	29.57	4.73	
		8 - days	13.48	64.15	0.09	1.96	4.51	28.2	4.51	
		12 - days	11.69	60.52	0.09	2.96	4.62	28.9	4.62	
		LSD		0.88	62.38	0.88	1.38	0.08	0.49	0.18
	TGX 199057f	Inoculated	4 - days	13.01	64.86	0.05	1.23	4.50	28.14	3.91
			8 - days	11.94	48.24	0.07	2.5	3.92	24.5	3.86
12 - days			11.92	71.08	0.05	2.08	3.90	24.35	3.87	
Non -inoculated		4 - days	12.15	65.01	0.07	3.41	3.66	22.9	3.66	
		8 - days	10.32	64.67	0.05	2.37	3.49	21.83	3.49	
		12 - days	8.72	59.27	0.03	4.89	3.44	21.53	3.44	
		LSD		2.25	66.52	2.25	1.37	0.11	0.69	1.38
TGX 198057		Inoculated	4 - days	14.96	69.05	0.26	3.14	6.11	38.16	6.1
			8 - days	14.2	63.98	0.07	3.05	5.83	36.46	5.83
	12 - days		13.05	64.88	0.05	3.56	5.78	36.1	5.78	
	Non-inoculated	4 - days	12.53	59.42	0.05	2.31	4.48	28.03	4.47	
		8 - days	11.44	50.26	0.06	2.79	4.49	28.04	4.49	
		12 - days	15.38	64.15	0.07	1.51	4.49	28.08	4.74	
		LSD		0.88	60.52	0.88	1.56	0.08	0.49	1.37
	<i>Stylozanthos</i>	Inoculated	4 - days	13.66	62.38	0.07	2.82	6.44	40.25	6.44
			8 - days	12.7	64.86	0.04	3.27	6.62	41.4	6.62
12 - days			11.7	48.24	0.07	3.17	6.65	41.56	6.64	
Non –inoculated		4 - days	12.67	71.08	0.12	2.01	7.69	48.04	7.68	
		8 - days	11.91	65.01	0.03	2.02	6.87	42.92	6.86	
		12 - days	13.63	64.67	0.07	1.67	7.67	47.92	7.66	
		LSD		0.68	59.27	0.68	0.18	0.11	0.69	1.56
<i>Desmodium</i>		Inoculated	4 - days	12.95	66.52	0.07	3.02	5.09	31.81	5.07
			8 - days	11.83	69.05	0.04	2.81	5.01	31.31	5
	12 - days		12.49	63.98	0.08	2.53	5.09	31.81	5.09	
	Non –inoculated	4 - days	12.87	64.88	0.08	2.07	5.09	31.84	6.32	
		8 - days	10.78	59.42	0.05	2.56	5.03	31.42	5.02	
		12 - days	11.28	50.26	0.04	2.64	5.01	31.34	5.01	
		LSD		6.45	64.15	6.45	1.98	0.11	0.66	0.48

Table 9. The leaf proximate and chemical composition of Legume species

	Water soluble carbohydrate (%)			Chlorophyll (mg/kg)			Ash (%)			Moisture content (%)			Crude fibre (%)			Crude protein (%)			Nitrogen (%)		
	4*	8*	12*	4	8	12	4	8	12	4	8	12	4	8	12	4	8	12	4	8	12
Legumes species	4*	8*	12*	4	8	12	4	8	12	4	8	12	4	8	12	4	8	12	4	8	12
<i>Phaseolus lunatus</i>	12.22 ^c	12.98	11.45 ^c	0.05 ^b	0.10 ^a	0.10 ^a	2.70 ^a	3.65 ^a	2.07 ^b	63.98 ^b	64.88 ^a	59.42 ^a	5.14 ^c	6.01 ^b	6.60 ^a	32.60 ^b	30.85 ^b	30.34 ^b	5.22 ^b	4.93 ^b	4.90 ^c
TGX 199057f	12.58 ^c	11.13	10.32 ^d	0.05 ^b	0.07 ^b	0.08 ^b	1.88 ^b	2.74 ^b	2.02 ^b	50.26 ^c	64.15 ^a	60.52 ^a	6.76 ^b	7.02 ^a	5.95 ^a	25.52 ^c	23.17 ^c	22.94 ^c	3.79 ^c	3.67 ^c	3.65 ^c
TGX 198057	13.75 ^a	12.82	14.21 ^a	0.06 ^b	0.15 ^a	0.05 ^b	3.19 ^a	2.58 ^b	2.32 ^b	62.38 ^b	64.86 ^a	48.24 ^b	6.73 ^b	6.83 ^b	5.63 ^a	33.09 ^b	32.25 ^b	32.09 ^b	5.28 ^b	5.16 ^b	5.26 ^b
<i>Stylozanthos</i>	13.16 ^b	12.3	12.66 ^b	0.05 ^b	0.09 ^a	0.05 ^b	2.53 ^a	2.79 ^b	2.24 ^a	71.08 ^a	65.01 ^a	64.67 ^a	7.01 ^a	7.70 ^a	6.85 ^a	44.14 ^a	42.16 ^a	44.74 ^a	7.06 ^a	6.74 ^a	7.15 ^a
<i>Desmodium</i>	12.91 ^c	11.31	11.89 ^c	0.07 ^a	0.03 ^c	0.06 ^b	2.67 ^a	2.87 ^b	3.77 ^a	59.27 ^b	66.52 ^a	69.05 ^a	6.93 ^b	6.21 ^b	6.76 ^a	31.82 ^b	31.36 ^b	31.57 ^b	5.69 ^b	5.01 ^b	5.05 ^b

4*, 8*, 12* (4-, 8- and 12- day watering intervals). Means along the column bearing the same letter are not significantly different by DMRT ($P = 0.05$)

The highest pod weight, seed weight number of seed per pod, number of nodule, nodule weight was obtained from inoculated species which was significantly higher than non-inoculated species. This result is in agreement with Shu-Jie et al. [39] who reported similar findings and concluded that the number of pods per plant, seeds per pod, and seed yield were significantly increased by different phosphorus levels. This may be attributed to the symbiotic relationship of rhizobia (bacteria) with the roots of leguminous crops, which fixes atmospheric nitrogen into the roots of legumes and thus the yield increased. This is in agreement with Stefanescu and Palanskiu [40] who reported that phosphorus and rhizobium inoculation induced a pronounced effect on yield. The authors inferred that number of pods per plant, seeds per pod, 100 grain weight and seed yield were significantly increased by different phosphorus levels.

The improvement of nodulation by rhizobium inoculation resulted in higher nitrogen fixation (Table 2) and consequently increased in vegetative growth yield of *Desmodium* when compared to the non-inoculated species. The results of present study are in conformity with observations made by Lawson and Quainoo [41-43] that non-inoculated plants produced decreased vegetative growth when compared to non- inoculated ones.

5. CONCLUSIONS

The effects of watering regime and rhizobial inoculation on the growth, nodulation and seed yield of some legume species: the staple; Legume Soybean (TGX 199057f and TGX198057) and forage legume species; *Stylozanthos* and *Desmodium* was evaluated. The legume species differed significantly in their responses to watering regimes and rhizobial inoculation based on the measured growth parameters. Seedlings that were watered at 4 and 8 days interval were more vigorous in growth compared to 12 days watering intervals. Rhizobium inoculated legumes produced more root nodule and number of leaves and taller plants compared with the non-inoculated species. The highest number of leaves and root nodules were produced by inoculated *Phaseolus lunatus*. Compared with 12- days watering interval, both inoculated and non-noculated seedlings subjected to 4- and 8- days watering intervals had higher root and shoot biomass. The non- inoculated TGX 199057f watered at 8- days intervals had the highest number of pods while

TGX198057 watered at 12 days had higher pod weight. Highest seed yield was obtained from soybean (TGX 198057) that were inoculated. *Desmodium* produced few seeds compared to *Phaseolus lunatus* and *Stylozanthos* across the watering regimes imposed. Rhizobium inoculation and 4- and 8- days watering intervals enhanced vigour of growth compared with 12- days watering. The effects of watering regimes and rhizobium inoculation varied on leaf proximate and chemical composition of legume species. The nitrogen, crude protein, ash, chlorophyll, soluble carbohydrates content in leaf differed among the legume species. The legume species inoculated and grown at 4- days watering intervals had the highest nitrogen content chlorophyll, soluble carbohydrates, ash, and moisture content compared to non-inoculated legume species. Application of rhizobium strain significantly increased seed yield of soybean and *Desmodium* and caused substantial increase in nodulation. Rhizobial inoculation will affect the N₂ fixation potential of the legumes under varying soil moisture regimes in the legume species. Based on the measured growth parameters in this study, rhizobium inoculation and 4- and 8- days watering intervals promoted plant vigour compared with 12- days watering interval. The study has shown that to increase legume productivity, application of rhizobium inoculant is recommended.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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