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Full Length Research Paper

Foliage productivity and quality of valuable medicinal plant (*Clitoria ternatea L*.) as affected by different fertilizers

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In order to investigate the productivity and forage quality of *Clitoria ternatea* under arid lands with poor soils, a field experiment was conducted to examine the effects of different fertilizers on forage productivity, quality, leaf area index (LAI) and stomatal conductance (g_s). Three types of fertilizers were applied; NPK (18-18-5+1.5 MgO), Urea (46) and Phosphate (DAP 18-46) at the rate of 90 tons/ha in three doses, in addition to the control. Dry and fresh yields (Tons/ha), were determine over five harvests. Leaves minerals (P, K, Mg, Na Ca), protein, fiber and ash contents were analyzed. LAI was measured by plant canopy analyzer and g_s was measured by leaf porometer. The results showed significant effects of fertilizers applied on yield, quality, LAI and g_s. Addition of fertilizers significantly increase dry and fresh forage yield, protein, LAI, minerals uptake, decreased fiber and ash compared to the control. NPK obtained the highest value in most of the parameters studied. There was strong relationship between LAI with forage productivity and quality. LAI also increased with an increase in plant growth. The results concluded that LAI can be a good estimate of plant productivity at different growth stages. Addition of NPK fertilizer improved forage productivity and quality over the control and other fertilizers.

Key words: LAI, stomatal conductance, forage productivity, forage quality, minerals.

INTRODUCTION

Clitoria ternatea L. (*Fabaceae*), known as Butterfly pea (Australia), Kordofan pea (Sudan) is a summer growing perennial climber, strongly persistent, multipurpose herbaceous legume. Due to its high values, the plant was naturalized in the semi-arid and sub-humid tropics in many countries (FAO, 2005). Recently many studies had indicated that *Clitoria* is very important medicinal plant species (Daisy and Rajathi, 2009). Ponnusamy et al. (2010) recorded that leaves extract of *Clitoria* has antimicrobial activity against fish pathogens, while Taur and patil (2011) revealed that roots of *Clitoria* exhibits antiasthmatic activity. The extracts from the roots enhanced acetylcholine in rats an indicator of memory enhancer (Rai et al., 2002). The plant is believed to be a good fodder species, due to its high forage palatability and preference by livestock over many legumes (Mukherjee et al., 2008). It is also, a good source of nitrogen supplement to grass basal diet (Juma et al., 2006). The ability of the plant to persist for many years under heavy dry season grazing on infertile soils (Hall, 1985) makes it suitable for the crop to be planted in waste lands (Michael and kalamani, 2003). It also exhibit good regrowth after cutting or grazing and can be used in agroforestry (Aguiar et al., 2009). *Clitoria* is proved to increase the levels of N, P and K in the soil (Alderete-Chavez et al, 2011).

Application of fertilizers in arid zone with saline soil under irrigated systems is very important to enrich soil nutrients, improve mineral uptake and reduce the deleterious effects of iron toxicity. Addition of phosphorus and nitrogen fertilizers to *Clitoria* resulted in improved growth, productivity and forage quality (Mohamed and Abu Suwar, 1996).

Many parameters can be used as indicators of plant growth and productivity. Among these parameters, leaf

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area index (LAI) and stomatal conductance (g_s) are the most important. LAI is a measure of total photosynthetic green tissues per unit ground surface area. LAI has strong relation with plant growth and development and positively correlated with addition of nitrogen fertilizers (Akmal and Janssens, 2004). In another hand, addition of phosphorus fertilizer increased the LAI of *Clitoria* (Abu Suwar and Omer, 2011). Stomatal conductance plays an important role in plant-atmosphere water exchange. It is the measure of the rate of passage of carbon dioxide and water vapour through the stomata of a leaf. Diffusion of CO_2 into the mesophyll of leaves and transpiration from the leaves to the atmosphere is mainly driven by the stomatal aperture, which is controlled by a complex system of plant physiological processes. Bahar et al. (2009), studied the relationship between stomatal conductance and yield in wheat, while Gomes et al. (2004), investigated the relationship between stomatal conductance and drought stress.

Saudi Arabia is laid entirely in arid and semi arid dry lands with very limited rainfall, low forest cover (FAO, 2009), and depleted grazing resources (Abo-Hassan and Tawfeeg, 2006). The future plan of the country is to limit growing water demanding forage crops due to depleted ground water reservoirs. Selecting persistent less water demanding plants may be the options. *Clitoria* could be one of the species to meet such requirements.

The objective of this study was to test the effect of addition of different nitrogen and phosphorous fertilizers on *C. ternatea* foliage yield and quality, leaf area index and stomatal conductance.

MATERIALS AND METHODS

Study area

The experiment was conducted at the Agricultural Research Station, King Abdulaziz University, at Hada Al-Sham during the period, November 2010/ December 2012. The Research Station is located about 110 km North-East Jeddah City (Latitude 21° 48' 3" N, Longitude 39° 43' 25" E, elevation 240 masl). The rainfall is very low (less than 100 mm/annum), the soil is poor with Ph ranging from 7.1 to 7.99 (Al-Solimani, 2003). The experiment was carried out under drip irrigation system.

Experimental design

The experiment design was a complete randomized block design with four fertilizer treatments and three replicates. The fertilizers applied were; NPK compound granular fertilizer (18-18-5 + 1.5 MgO, Granfield-Fort), Urea (46, Sabic), Phosphate fertilizer was added in form of Diammonium Phosphate (DAP 18-46-0, Sabic), plus control untreated. For all of the three types of fertilizers a rate of 90 tons/hectare were added at frequency of three doses. Viable seeds of *Clitoria* were directly sown in rows at spacing of 40 × 50 cm (giving 50,000 plants per hectare). A plot of four rows, 10 m long was planted for each treatment in each block. The plants were allowed to grow normally for three months, after that the first cut was made, followed by other subsequent harvests at frequency of 6 weeks between the cuts. A total of 5 cuts were harvested. The plots were divided into two section; section one the plants were cut, whereas the plants in section two were allowed to grow in which leaf area index and stomatal conductance were measured at different growth development.

Clitoria yield

Clitoria crops were cut at 6 weeks interval for five consecutive cuttings. Dry and fresh crop yields tons/hectare was then estimated for each of the five harvests. In addition to the total yield (sum of all harvests).

Mineral contents

Leaves mineral contents (Ca, Na, K, P and Mg) were analyzed using Perkin Elmer model 3110 atomic absorption spectrophotometer (PERKIN ELMER CORP, 1994), according to Hanlon (1998), while P was determined according to the method described by Bhargava and Raghupalhi (1993).

Protein content

Protein content was estimated as nitrogen percent multiplied by 6.25. Nitrogen was analyzed by micro-kjeldah method (Horneck and Miller, 1998).

Crude fiber and Ash contents

The crude fiber and ash contents were determined according to the AOAC official methods 978.10 and 942.05, respectively (AOAC, 2006).

Leaf area index (LAI)

LAI, which was considered as foliage area index, because the green upper parts include leaves and small branches and twigs, was measured by plant canopy analyzer (model, 2200, LI-COR Biosciences, 2011). The sampling protocol used was that having one reading above for every four readings below. The four readings below were made between rows (one at the right side of the row, two in the middle of the row and the fourth at the left side of the row). A total of three measurements were made at frequency of every three weeks to measure plant growth development. Mean LAI for each plot plus SEL (standard error of LAI) was directly loaded from analyzer console using (FV 2200 ver. 1.0.0) software.

Stomatal conductance (g_s)

Stomatal conductance was measured with steady state leaf porometer (Leaf Porometer upgraded model SC-1, 2011, Decagon Devices). For each treatment in each block; three plants were selected and five leaves were measured per plant. All the measurements were made in the morning between 09:00 to 11:00 o'clock. Fully grown green leaves at the same side for all sampled plants were measured. A total of three measurements at three weeks interval were made.

Statistical analysis

Analysis of variance (ANOVA) was carried out to test the effects of the main treatments and the means were separated by Duncan multiple range test (El-Nakhlawy 2010). [Statistical analysis system

Table 1. Effect of nitrogen and phosphate fertilizers on dry yield (Tons/hectare) of *C. ternatea*.

Treatment	Harvest 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5	Total yield
NPK	3.80[a]	2.48[ab]	1.71[a]	1.67[ab]	0.96[a]	10.62[a]
Urea	2.70[b]	2.44[ab]	1.72[a]	1.84[a]	0.69[b]	9.40[ab]
Phosphate	2.27[b]	2.86[a]	1.11[a]	1.53[ab]	0.63[b]	8.41[bc]
Control	2.31[b]	1.84[b]	0.92[a]	1.15[b]	0.67[b]	6.89[c]
P=	**	*	Ns	*	**	**
Significance	* = ≤ 0.05		** = ≤ 0.01		ns = not significant	

Table 2. Effect of nitrogen and phosphate fertilizers on fresh yield (Tons/hectare) of *C. ternatea*.

Treatment	Harvest 1	Harvest 2	Harvest 3	Harvest 4	Harvest 5	Total yield
NPK	12.31a	5.54ab	3.78a	4.60a	2.89a	29.12a
Urea	7.99b	6.11a	3.61a	5.11a	2.31ab	25.13ab
Phosphate	6.57b	6.71a	2.33a	3.76a	1.93b	21.31bc
Control	7.23b	4.01b	1.76a	2.78a	2.06ab	17.85c
P=	**	*	ns	Ns	*	**
Significance	* = ≤ 0.05		** = ≤ 0.01		ns = not significant	

(SAS), SAS System version 8.1, 2000] package was used for data analysis.

RESULTS

Forage yield

ANOVA results showed that dry yield production was significantly differed between the different fertilizers applied (Table 1). Higher dry yield was obtained in NPK fertilizer, where it was produced more that 10 tons of dry matter over the five harvests. In turn urea produced higher dry matter than phosphate. The control showed the least dry yield production. Dry yield production was decreased with harvesting, whereas it was highest in harvest one and the least in harvest five. However, harvest three produced relatively less dry matter than harvest four. This is because; harvest three came at the very hot summer time.

Similar to dry yield, fresh yield production was differed and significantly affected by the fertilizer type applied, where NPK had high fresh yield production (Table 2). Although the general trend was decreasing of fresh matter with harvesting time, the exception was harvest three which produced less dry matter than harvest four. This also, was attributed to the hot summer season.

Forage quality

Protein, fiber and ash production was significantly differed with the different fertilizer types applied (Table 3). The protein contents in *Clitoria* leaves ranged from 21 to 28%, with higher protein content produced as the result of NPK fertilizer application. The fiber content ranged between 14 to 15% and the ash ranged from more than 9 to more than 10%.

Minerals contents

The results revealed that addition of fertilizers resulted in significantly higher mineral contents (Table 4). Apart from N, all minerals contents were significantly higher in plots treated with NPK fertilizer.

Leaf area index

The results shown in Figure 1 revealed significant effects of fertilization on LAI. There is strong relationship between LAI with amount of forage yield and quality. The highest LAI was produced as the result of NPK addition. The results showed an increase in LAI with increase plant growth. In measurement one, the LAI was ranged from 6.86 for NPK to 4.21 for control, in measurement two, it was ranged from 6.94 for NPK and 4.53 for control, whereas in measurement three it was from 8.47 to 6.68 for NPK and control, respectively.

Stomatal conductance

Results obtained in this study revealed relatively high values of g_s over all the treatments. The high conductance was obtained as the result of phosphate and NPK

Table 3. Effect of nitrogen and phosphate fertilizers on protein, ash and fiber contents (%) of *C. ternatea*.

Treatment	N	Protein	Ash	Fiber
NPK	4.5a	28.0a	9.76d	14.16c
Urea	4.1b	25.38b	10.15c	14.59b
Phosphate	4.4a	27.46a	10.47a	14.48bc
Control	3.3c	21.52c	10.39b	15.80a
P=	**	**	**	**
Significance	* = ≤ 0.05	** = ≤ 0.01	ns = not significant	

Table 4. Effect of nitrogen and phosphate fertilizers on minerals (Mg, Ca, Na, K) contents of *C. ternatea*.

Treatment	Mg (mg/L)	Ca (mg/L)	Na (mg/L)	K (mg/L)	P (%)
NPK	431.6a	332.7a	79.8b	224.1a	0.36a
Urea	427.1ab	200.0c	85.5a	187.7b	0.33a
Phosphate	424.7b	245.8b	27.1d	174.4c	0.36a
Control	424.9b	245.3b	34.5c	171.7d	0.37a
P=	*	**	**	**	Ns
Significance	* ≤ = 0.05	** ≤ = 0.01		ns = not significant	

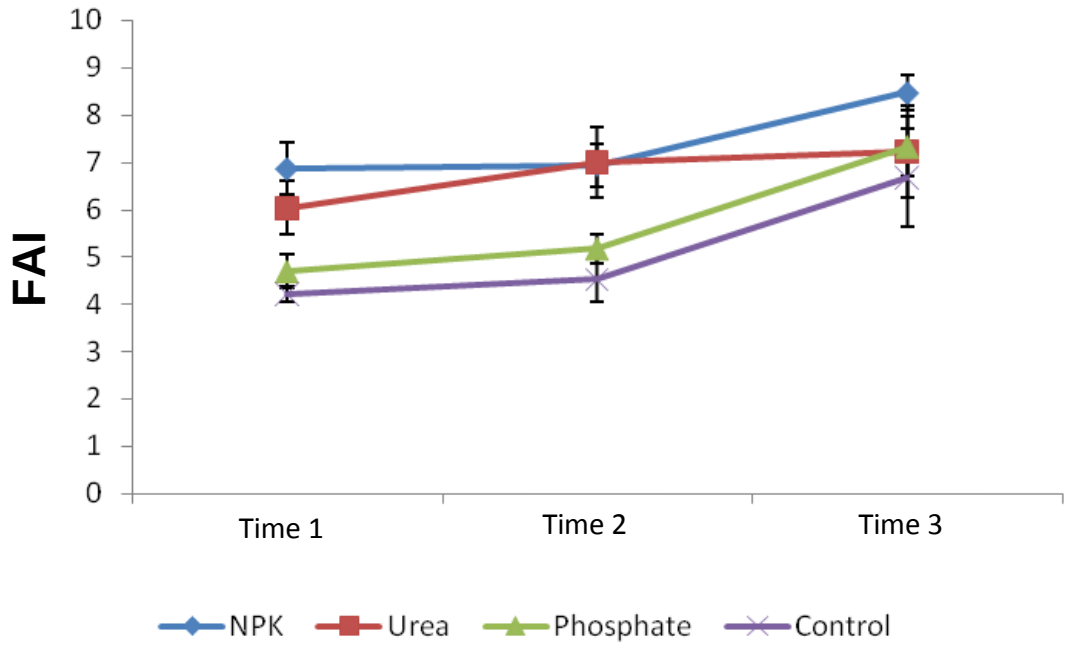

Figure 1. Mean Leaf area index (LAI ± Std.err) of *C. ternatea* as affected by different fertilizers applied, measured by Plant Canopy Analyzer (LI-COR Biosciences) for three times at three weeks interval.

fertilizers addition. g_s ranged from 446.6 mmol $m^{-2}s$ for urea to 644.9 mmol $m^{-2}s^{-1}$ in phosphate for measurement one. In measurement two it was from 452.9 mmol $m^{-2}s$ in control to 667.5 mmol $m^{-2}s$ in NPK, while in measurement three it was from 488.5 mmol $m^{-2}s$ to 644.9 mmol $m^{-2}s$ for urea and phosphate, respectively (Figure 2).

DISCUSSION

Forage yield

The results of current study revealed a very high effect of fertilizer application on plant productivity under arid dry

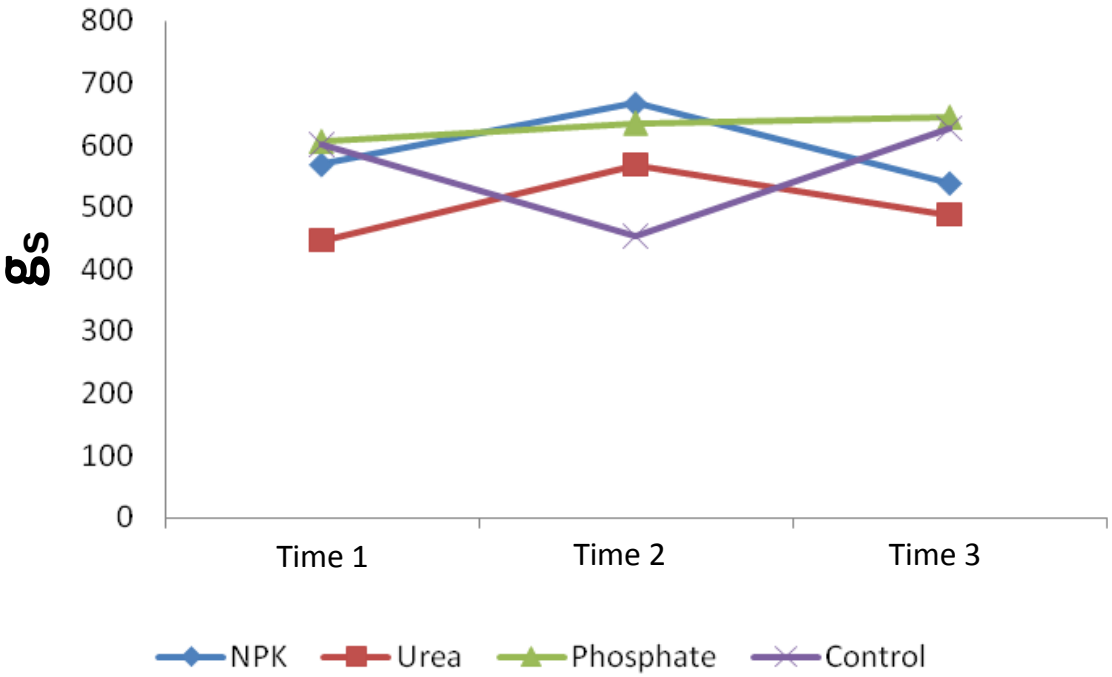

Figure 2. Effect of fertilizers application on stomatal conductance (g_s, mmol $m^{-2}s^{-1}$) of *C. ternatea* as, measured by steady state leaf porometer (SC-1, Leaf Porometer, Decagon Devices) at different growth stages.

conditions. Application of NPK fertilizers significantly increased productivity, followed by pure nitrogen in form of urea. Nitrogen fertilization tends to increase many parameters that have positive contribution to dry matter yield and quality (Carpici, 2011). The forage production decreased with increasing cutting time. However, harvest three showed lower production than harvest four. Harvest three came during the very hot summer season. This reflects the seasonality effects in productivity. The fresh yield production was very high (29.1 to 17.9/ tons/ha), compared to dry yield (10.6 to 6.9). The high performance of NPK addition, increase the availability of the N, P, K in the soil, resulting in better growth and forage yield.

Forage quality

The crude protein content ranged from 28.0 to 21.5%. This is close to Abu Suwar and Omer (2011), who reported a crude protein content of 16.6 to 28.3%. However, fertilizer treatments had high effect on crude protein. NPK and phosphate produced the highest protein content, followed by urea. The same trend was also, showed for fiber content. It is believed that addition of nitrogen fertilizer increase protein, while decrease crude fiber, which could increase digestibility and palatability (Almodares et al., 2009). The same trend was also true for phosphorous fertilizer application (Abu Suwar and Omer, 2011). Although, the fertilizers types significantly differed in ash contents, the range was between 9.76 to 10.47%. According the hay forage growers (hayandforage.com), a range of 6 to 9% of ash content in the ration is probably a good benchmark to use, because higher contents of ash means that a lot of potential contamination or dirt materials available in the forage. Our results showed that application of fertilizers increased minerals contents in leaves. NPK application increased (Mg, Ca, K) uptake in plant leaves. The increase of forage yield and nutrient uptake was reported by (LI et al., 2010).

Leaf area index

The leaf area index LAI is a total photosynthetic green foliage per unit ground surface area. The theory of the plant canopy analyzer depends on the measurement of photosynthetically active radiation (PAR). This means that there is indirect relation of LAI with plant production. Our results revealed a significant effect of fertilizer application on LAI. In the same way there is a very strong relationship observed between LAI and forage yield and quality. The increase of LAI with increased plant growth and nitrogen addition was reported by Akmal and Janssens (2004), where Carpici (2011) revealed positive association of LAI with phosphorous application. The positive correlation of LAI with forage productivity indicates

that LAI can be used as indirect measure for yield estimation.

Stomatal conductance

The present study showed that stomatal conductance was generally high among all treatments. Although g_s was higher under phosphate and NPK fertilizers application, there was no consistent relationship of stomatal conductance with forage productivity. This is similar to Bahar et al. (2009), who found negative correlation between g_s and wheat grain yield and positive correlation with some growth attributes. We believed that the variation in individual measurements of g_s values could be attributed to variation in weather conditions, especially humidity that is fluctuating frequently in this area. Also, we observed a very high variation in leaves RH% and sensor filter RH% between individual observations, as measured by the instrument, though we tried to reduce the variation to minimum by maintaining the measurement time to the minimum, using the same size of leaves and measuring in the same direction. Gomes et al. (2004), showed similar conclusion when they studied stomatal conductance on orange trees that were submitted to drought stress.

Conclusion

Under arid land poor soils, addition of fertilizers significantly increased forage productivity and quality and minerals uptake. There is positive relationship of LAI with forage productivity and quality. Addition of NPK improved crop productivity and quality over the other treatments.

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