Sudan University of Science and Technology

College of Graduate Studies

Effect of Rhizobium, and Monoammonium Phosphate Fertilizer on the Growth and Forage Yield of Clitoria (*Clitoria ternata*)

تأثير باكتريا العقدين وسماد أول أمونيم الفوسفات على نمو وحاصل علف الكلايتوريا

A Thesis Submitted to Sudan University of Science and Technology in Partial Fulfillment for the Requirements of the Degree of M.Sc.in Agronomy

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الآية

قال تعالى:

(أَفَرَأَيْتُمْ مَا تَحْرُثُونَ * أَأَنْتُمْ تَزْرَعُونَهُ أَمْ نَحْنُ الزَّارِعُونَ)

صدق الله العظيم

سورة الواقعة الآيات (63-64)

Dedication

To my dear mother and my dear Father

To my sisters and brothers

To all my teachers and colleques

And finally to all my friends

and all Relatives for their kind help and support.

And to soul of my dear sister Ehlam

Rehab

.

Acknowledgement

Most grateful thanks to Allah for giving me health, patience, and assistance to complete this work.

Thanks and all thanks to my supervisor, **Dr. Sami Ali Mohamed Hamid** for supervising and nice cooperation and help.

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Abstract

To study the effect of mono ammonium phosphate and rhizobium bacteria on the Growth and yield of *Clitoria ternata*. (L). a field experiment was carried out in the summer season of 2016 at the demonstration farm of the College of Agricultural Studies, Sudan University of Science and Technology Shambat, for this experiment a randomized complete block design was used with three replications, the treatments were Control (C), Inoculum (I), 100kg map/ha(MAP), Inoculum+100kg MAP/ ha (1+100 MAP), Inoculum +50kg MAP/ ha (1+50 MAP).

The result showed that treatments exceeded the control in all attributes evaluated, but no significant difference among these treatments in the attributes evaluated.

ملخص البحث

لدراسة تأثير أول فوسفات الأمونيوم وبكتريا الرايزابيوم على نمو وإنتاجية محصول الكلايتوريا صنف بلدي أجريت التجربة الحقلية في الموسم الصيفي للعام 2016 في المزرعة التجريبية بكلية الدراسات الزراعية – شمبات – جامعة السودان للعلوم والتكنولوجيا – لهذه التجربة أستخدم تصميم القطع العشوائية الكاملة في ثلاثة مكررات المعاملات هي: الشاهد، الرايزوبيوم، 100 كيلوجرام مونو امونيوم فوسفات/ هكتار، 100 كيلوجرام أحادي فوسفات الأمونيوم + رايزوبيوم، 50 كيلوجرام أحادي فوسفات الأمونيوم + رايزوبيوم.

النتائج لم تظهر أي فروقات معنوية بين هذه مقيمة للمعاير التي قيمت هذه المعاملات.

CHAPTER ONE

INTRODUCTION

Kordfan butterfly pea (*Clitoria ternatea* L.)belongs to the family Fabaceae. Genus clitoria consists about 30 species which spread in all warm places in the world. Species *C .ternata* is one of this species which produced high quantity of the good forage from natural pastures and irrigated pastures. This forage crop is adapted to dry tropical lands, because it is used as a green manure. Sudan is rich in animal resources .Sudan has 102 .846.331 heads of cattle, sheeps, goats, and camels (ELawad ,2011) . This huge animal resource contributes very much to the national economy. Despite this large number of animals, the range conditions of the country is poor and very much deteriorated to cope with animal needs. This necessitates that irrigated forage need to be given at priority in the irrigated sector to bridge the gap between forage supply and animal feed demands .Irrigated forages contribute only about 4% of the total forage available to livestock in the Sudan.

Among the promising forage crops that could receive more attention is *clitoria*, which is also known as Kordofan butter fly bean .This crop can be grown both by rain and under irrigation. It is a high yield and drought tolerant .it is nutritive value is not much different from that of alfalfa. It contains 14to22% crude protein, 9 to 12% digestible protein and 56% total digestible nutrients (TDN)(Cook at 2005).

The potential area for growing clitoria either irrigated or non- irrigated in the central clay plain .The central clay plain soils are deficient in nitrogen and phosphorus. As a legume clitoria is capable of symbiotic N_2fixation .Phosphorus is needed for better nodulation process as a source of energy in the form of adenosine tri phosphate (ATP).(ECO CROP 2012).

The objective of this study is to evaluate the effect of the Rhizobium inoculums and the mono ammonium phosphate fertilizer on:

1. The nodulation,
2. growth rate, and,
3. the forage yield of Clitoria

CHAPTER TWO

LITERATURE REVIEW

2.1. Botany

The butter fly pea (*Clitoria ternate(* L), is vigorous twining , scrambling or climbing tropical legume, it is sparsely pubescent. Stems are sub erect and woody at the base and may be up to 1m long .the root only at the tips, Cook *et ,al*, 2005). The leaves are pinnate, bearing 5-7 elliptical, 3-5 cm long leaf lets. The flowers are solitary or paired, deep blue or pure white about 4 cm board. The fruits are flat, linear, sparsely pubescent pods that dehisce violently at maturity and throw 8-10 dark and shiny seeds (FAO (2012). There are numerous ecotypes –agro- types and cultivars that differ in flowers and leaf lets (FAO 2012). Many cultivars have been bred in Latin American, notably in Cuba and Mexico (Cook, *et, al* (2005).

2.2. Economic Importance

A nitrogen fixing legume, *Clitoria ternata*, is used as a green manure ,it is avaluable cover crop in rubber and coconut plantations, the young pods are edible and used as vegetables in Philippines (Staples 1992), Butter fly pea is used in fences as an ornamental for its showy flowers, valuable for dyeing and ethno – medicine (Cook *et al* 2005).

2.3. Distribution

Clitoria ternate may have originated from Latin America or Asia but is now naturalized in all semi and sub humid tropics of Asia, Africa, and Australia (Staples-1992).*It* grows within 200 and 240 m form sea level up to an altitude of 800-1600 m ,and in equatorial Africa up to2000 m (FAO, 2012).

2.4. Ecology

2.4.1. Climate

2.4.1.1. Temperature

Clitoria does better where average temperature is about $19C^0$-$28C^0$and where rain fall ranges from 700 to1500mm, however it tolerates temperatures as low as 15°C and even some frost as it may re grow from the stems of the plant base, provided it is already woody when the frost occurs (ECO Crop, (2012).

2.4.1.2. Rainfall

It does well under Irrigation but has only low tolerance of flooding or water logging, it has also som-e, drought tolerance and can grow in places where rain fall is as low as 400-500 mm, it can survive a 5-6 month drought in the drier tropics (Cook *et al*, (2005).

2.4.1.3. Photo Period

It thrives in full sunlight, but can also grow under light shade in rubber and coconut plantation (Staples, (1992).

2.4.2. Soils

Clitoria can grow in a wide range of soils, but is a particularly adapted to shallow, heavy clay and sodic soils of pH 5.5 - 8.9 (FAO, 2012).

2.5. Cultivars

Over 110 accessions of *Clitoria ternate* have been introduced to Australia from some 24 countries. Reid and Sinclair (1980) described the morphological and agronomic variation of 55 of these plus three naturalized when grown as spaced plants at Land down Research station, Australia. In Sudan land races are grown.

2.6 Cultural practices

2.6.1 Land Preparation

To prepared the land by the disc plough, Disc harrow, leveling, Ridding, Plantation In hole, 15 cm between the hole and other. (Abuswar, 2004).

2.6.2 Sowing date

July is the suitable sowing time, but high production can be obtained when sown in March, (Khair, 1999).

2.6.3. Irrigation

In autumn season irrigation is every 12 days, with 300 mm//feddan, of water. When clitoria planted at March ,it is irrigated with 350 mm/feddan every 12-14 days. *Clitoria ternatea* is drought resistant crop, (Abuswar, 2004).

2.6.4 Weed control

In early stages of growth clitoria is sensitive to weed, competition. ,In gezira scheme, weed control is achieved by hand twice, before the crop establishment .In the Rainfall reigons the weed control is achieved by Pre Ploughing. (Khair, 1999).

2.6.5 Diseases and Pests

Clitoria is effected by musaic virus and stem rot. The Pests that effect, clitoria effected by the thrips, the diseases and pests have no economic importance. (Abuswar, 2004).

2.6.6 Fertilization

Clitoria is anitrogen fixing crop, so it needs starter dose (1N)at sowing time. Clitoria response to phosphorus which increased yield (Ibrahim et.al 1996 and Khair,1999).

2.6.7 Harvesting

In October harvest carried out for the crop which planted in July, the second cutting is at January. In the rainfall reigon harvest time last January grazed .. (Abuswar, 2004, and Khair, 1999).

2.7. Nutrition

2.7.1. Mineral Nutrition

2.7.1.1. Nitrogen

Nitrogen is one of the most important nutrient element affecting the yield, the legumes is known to be highly nitrogen demanding crop , since the end product is very rich in protein . T he main source of nitrogen for the crop so as to achieve high yield is the sym biotic N2 fixation . that the crop can obtain a large protein of this nitrogen from atmosphere through its symbiotic relationship with rhizobium bacteria (Singh and Saxena, 1972). Nitrogen fixation begins ten days after planting , only when plants were grow under optimum moisture and temperature conditions starter amount of nitrogen at planting could be beneficial of early growth .

Boroomudan *et al* (2009) and, Darrel *et al* (1989) stated that large amounts of nitrogen a rend applied because of the ability of legumes to fix atmosphere nitrogen , but in some areas complete starter fertilizer .such as 6-24-24 t/ha containing small quantity of nitrogen is used at planting .The nitrogen can stimulate early vegetative growth.

2.7.1.2. Phosphorus fertilizer

Wild (1988) and Russel (1973) reported that low up take can be a consequence of:

(I) adding the fertilizer to the soil of high phosphate status.

(II) plant growth limited by some factors oher than phosphate supply.

(III) inaccessibility of phosphate to root for some reasons .and ,

(IV) the adsorbed phosphate being converted in to non valuable form.

Mohammed (1994) reported that phosphors applied considerably increased growth parameters plant density,, plant, height,, fresh and dry forage,, leaf to stem ratio, and leaf area index, m alfalfa. Mohammed (1990) pointed that application of phosphorus fertilizer significantly increased forgae firesh weight, and dry matter of forage legumes and grasses. Ahmed (1988) reported that phosphatic fertilizer whether applied as whole or split does did not effect vegetative and reproductive attributes. (Abdallah (1999) repotted that application of phosphors increased plant density, leaf area, leaf to stem ratw, fresh weight and dry weight of clitoria. He also found that application of 50 kgP_2O_2/ha resulted in a higher plant cover and increased yield of clitoria. Zaroug (1978) stated that in clitoria number and size of nodules were reduced by deficiency of sulphur or phosphorus. Sulphur has more favorable effect on this attribute than phosphorus.

Fahmina *et al* (2013) tested four levels of P_2O_2 (viz 0,15,30,50, kg p2. /ha) and reported that application of different levels of phosphorus showed that plant height and number of branches per plant increased up to 50 kgp_20_5/ha. As well biological yield increased significantly up to 30 kg $P_2.O_2$/ha Mahmoud *et al* (2009) observed that phosphorus application increased plant height, leaf area, Number of branches, crop, relative growth rate, number of pods per plant, of soybean. Mahmoud *et al* (2009) found that application of 30kgp_2o_5 t /h produced significantly higher number of branches than at $05p_2o_5$ t //ha. Mahmoud *et al* (2009) showed that phosphors application increased the root length ,density , shoot dry weight per plant, leaf area index, number of pods per plant, and 100 seed weight of soybean.

2.7.2. Biological Nutrition

In symbiotic relationship carbohydrates and minerals are supplied to the bacteria by the plant, and the bacteria transform nitrogen gas from the atmosphere into ammonia NH3 for use by the plant (Hiati,1993) Ibrahim *et al* (2011) stated that at Shambat. inoculation significantly improved the dry weight of shoots and roots, nodulation, yield and yield component. Papastyliana (1986) showed that nodules were only formed on Inoculated plants and their number, varied with inoculums.

2.7.3. Interaction Between Mineral and Biological Nutrition

Majid (2009) stated the effect of rhizobium inoculation and phosphors fertilization on nodulation, growth and yield characters of soy bean grown in the presence of starter N fertilizer and recoded that rhizobium inoculation and phosphorus increased number of nodules over uninoculated control. Ibrahim *et al* (2011) showed that inoculation increased plant height, nodulation, number of pods/plant, 100 seed weight and gave seed yields of 1.43,1.41,1.59 t/ha respectively compared with 1.41 t/ha ,for the without inoculation or urea.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental site

A one season experiment was conducted on summer season of 2016 at the Demonstration farm of College of Agricultural Studies, Sudan University of Science and Technology at Sham bat. Sham bat is located at latitude 15^{o}-40\ longitude 32^{o} 35\, and ultitude 280 m sea level , , within the semi desert climate described by Adam (2003) Appendix l. The soil of site is clay with pH 8.2 as described by Abdelgadir (2010) Appendix 2.

3.2.1. Plant Material

Land race of Kordfan pea. *Clitoria ternata* L.

3.2.2 Inoculum *Rhizobium phaseoli*

Strain: Tal 1282, obtained from National R esearch Centre, Khartoum.

3.2.3. Fertilizer

Mono Ammonium phosphate (MAP) $NH_2H_2PO_4$; 12%N 61%P_2O_5.

3.3 Methods

3.3.1. Land Preparation

The land was prepared by plughing, harrowing, leveling, and ridging at 70cm apart then divided into plots each of 4 / 3.5m long ,area the plot size is 14m^2. 2.

3.2.2. Experimental Design

Randomized complete block design (RCBD)with three replicates.

3.3.3.Treatments

- Control (C).
- Inoculums (I).
- 100kg MAP/ha(MAP).
- Inoculum+100kg MAP/ ha (1+100 MAP).
- Inoculums +50kg MAP/ ha (1+50 MAP).

3.3.4. Sowing

Sowing was carried out on01 /08 /.2016. 2-3 seeds were sown per hole, in intra-row spacing of 20 cm.

3.3.5. Stand balance

The stand balance (thinning + resowing) was carried out one week after emergence. Only two emerged plants were left per hole.

3.3.6. Fertilizer application

The MAP fertilizer was applied before sowing.

3.3.7. Weed control

Weeds were controlled manually twice ,the first weedings 2 weeks and the second weed 4weeks after sowing.

3.3.8. Irrigation

The plots were irrigated every 10 days.

3.4 Data collection and analysis

3.4.1 Data collection

3.4.1.1. Relative growth rate (RGR)

Half running meter length was taken from the middle rideg of each plot two times on 28/.08 and 08/09/2016. the taken samples were oven dried weighed, and the dry weights were recorded to calculate the RGR according to Radford (1967) as follows:

$$\frac{(DW2 - DW1)}{A \times E}$$

Where A = area

., And T = time (in g.m./ day).

3.4.1.2. Nodulation

The root were taken at the 50% flowering stage, and the number of nodules was counted (mean of three plants taken randomly from the middle of two ridges of each plot).

3.4.1.3. Forage fresh weight

One runing meterlength from one of the middle ridges (i-e 0.7 m^2) of each plot was harvested, and the yield per area was calculated.

3.4.1.4. Dry weight

One kilogram from the fresh weight harvested above was taken, oven dried at 70 C^o for 48 hours to calculate the dry weight percentage per each plot.

3.4.2. Statistical analysis

The analysis of variance (ANOVA) was worked out for every character. Duncan multiple range test (DMRT) was then used to seperate the means according to Little and Hill (1978).

CHAPTER FOUR

RESULTS

4.1 Number of Nodules per plant

The inoculums ,and inoculums+100kg map/ha treatments resulted in the heights number of nodules per plant, which the treatment of mono ammonium phosphate resulted in the lowest number of nodules per plant . In all the treatments there were not significant differences .(Table 1).

Table 1. Number of Nodules per plant

Term	**Nodules/ plant**
CONTROL	23.1[a]
INOCULUM	23.9[a]
INOCULUM+100kg MAP/ha(1+100	23.9[a]
INOCULUM+50 kg MAP/ha(1+50	20.2[a]
MAP	17.2[a]
C.V%	60.42

Means with the same letter (s) within the column are not significantly different at 0.05 probability level according to Duncan Multiple Range Test (DMRT).

4.3 Relative growth rate

In this measurement the highest result registed in control treatment, the second one in inoculum treatment and inoculums +100 kg MAP /ha, the third one in inoculums + 50 kg MAP / ha treatment, the fourth one in MAP treatment,

Table 2. Relative growth rate

Term	R.G.R ($gm/m^2/day^{-1}$)
CONTROL	8.5^{a}
INOCULUM	6.4^{ab}
INOCULUM+100 kg MAP	6.4^{ab}
INOCULUM+50 kg MAP	2.7^{ab}
MAP	0.49^{b}
C.V%	50.8

Means with the same letter (s) within column are not significantly diferent at 0.05 probability level according to Duncan Multiple Range Test (DMRT).

4.2 Fresh Weight

All treatments produced the same fresh weight (Table 2).

Table 3. Fresh Weight

Term	Fresh Weight Kg /m$^{2)}$
CONTROL	2.3^{a}
INOCULUM	2.3^{a}
MAP	2.3^{a}
INOCULUM +100 kg MAP	2.3^{a}
INOCULUM +50 kg MAP	2.3^{a}
C.V%	20

Means with the same letter (s) with in column are not significantlydiferent at 0.05 probability level according to Duncan Multiple Range Test (DMRT).

4.4 Dry weight

The treatment of inoculum + 100 kg MAP/ ha produced the highest dry weight, where the control produced the lowest. The differences among all treatments were not statistically significant (Table 4)

Table 4. Dry weight

Term	Dry Weight (%)	Fresh Weight Kg /$m^{2)}$
CONTROL	20.6[a]	2.3[a]
INOCULUM	26.6[a]	2.3[a]
INOCULUM+100 kg MAP	29.0 [a]	2.3[a]
INOCULUM+50 kg MAP	23.3 [a]	2.3[a]
MAP	24.0 [a]	2.3[a]
C.V%	17.6	20

Means with the same letter (s) within column are not significantlydiferent at 0.05 probability level according to Duncan Multiple Range Test (DMRT).

CHAPTER FIVE

DISCUSSION

The results revealed a reasonable number of nodules per plant in all treatments including the control treatment without significant differences among them. This could be attributed to the presence of strain that inoculates the clitoria in the soil, that clitoria been grown before on the soil of the site .However, the nodules size is very small i.e not well developed to fix the nitrogen .It is reported that supply of phosphors is very important for the development of nodules as a source of energy in the form of ATP, Hiati (1999).

It has been expected that mono ammonium phosphate ; since it contains higher percentage of $P_2 O_5$than the triple super phosphate, to provide phosphors to clitoria plants. This was mainly due to the fact that Shambat soil; as central clay plain is diffident in available phosphorus as reported by Dawelbeit *et al* (2007). They added that such soils are characterized by relatively high cation exchange capcity as well as base saturation ratio that make the respons to application of in organic fertilizer. This was supported by Ibrahim *et al* (1996), that clitoria responded well to the application of phosphours as triple super phosphate under the condition of the central clay plain .Not only this ,di ammonium phosphate as an other source of phosphors was reported to increase the growth and yield of clitoria, (Ali ;*et al* , 2007 a); and Whael (Ali *et, al* 2007 b) grown in soil of central clay plain.

Mono ammonium phosphate contains ;as the label indicates ,61%$p_2o_5$but failed to increase the growth and the forage yield of clitoria . This compared with triple super phosphate (46% P_2O_5) A. well diammonium phosphate (46%P_2O_5) where both increased the yield of and other crops grown in alkaline clay soil of central clay plain . The 12% N of the fertilizer was not

enough to substitute the biological N_2fixation to increase the growth and forage yield of clitoria.

CHAPTER SIX

SUMMARY AND NCOCLUSION

6.1. Summary

To evaluate the compact of rhizobium and mono ammonium phosphate on nodulation; growth and yield of clitoria forage, a one season field experiment was carried out at Shambat in the summer season of 2016 . The experiment consisted of control, inoculum, 100 kg map /ha, inoculum +100kg map /ha ,inoculums +50kg map/ha treatments .The treatments were layed out within a complete randomized block design with three replicates. The results reavled no significant differences in number of nodules per plant ,relative growth rate ,and dry weight among all treatments.

6.2. Conclusion

According to results, it could be concluded that mono ammonium phosphate availability at the alkaline clay soil of Shambat is very low, this was resulted in ;

1. very poor nodulation, and N_2 fixation.
2. no increase in the relative growth rate, and henc,.
3. no increase in the forage yield of clitoria.

Further research on nitrogen and phosphorus nutrition of clitoria is strongly recommended .

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APPENDICES

Appendix 1; The semi – desert climate ;

Sun shine duration 3650 hour /year .

Solar radiation 22.7 $MJ/m^{2/day}$.

Maximum temperature 42 c^{o} (may)

Minimum temperature 12 c^{o} (January)

Temperature range 30 c^{o}.

Rainfall 100- 250mm/annum

Evaporation 2400mm/annum.

Appendix2: Chemical and Physical Properties of the filed soil:

PH	8.0
ECC ds/m	1.7
SAR	6
Soluble Cation(meg/1)	
Ca+Meg	09
Na	1.0
K	0.2
CLmeg/L	0.8
N%	0.08
P.p.p.m	7
$CaCo_3$%	2.00
Sand%	37
Silt%	15
Clay%	48

3.1 Number of Nodules

Source	DF	SS	MS	F	P
TREM	4	380.25	95.062	0.52	0.7251
Error	10	1837.01	183.701		
Total	14	2217.25			
Grand Mean 22.433		CV 60.42			

3.2 Relative Growth Rate

Source	DF	SS	MS	F	P
TREM	4	119.793	29.9482	5.19	0.0158
Error	10	57.651	5.7651		
Total	14	177.444			
Grand Mean 4.7187		CV 50.88			

3.3 Fresh Weight

Source	DF	SS	MS	F	P
TREM	4	0.00000	0.00000	M	M
Error	10	0.00000	0.00000		
Total	14	0.00000			
Grand Mean	2.3000	CV	0.00		

3.4 Dry Weight

Source	DF	SS	MS	F	P
TREM	4	122.933	30.7333	1.62	0.2446
Error	10	190.000	19.0000		
Total	14	312.933			
Grand Mean	24.733	CV	17.62		