

Effect of Dual Inoculation with Rhizobium Bacteria, A- Mycorrhizal Fungi and Micronutrients on Productivity of Egyptian clover

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ABSTRACT: Two field experiments were conducted at the Experimental station Farm of Facility of Agriculture (Saba- Basha). Alexandria University. Egypt. During 2014/2015 and 2015/2016 growing seasons. The objective of this study was the investigate the effect of dual inoculation with Rhizobium & A- mycorrhizal fungi and micronutrients on productivity and quantity of Egyptian clover (*Trefolim alexandrinum*, L.) (cv. Giza 6). The obtained results recorded that summarized as follows. Application foliar micronutrient (Ca +B) at two times sprays gave the highest significantly effect on growth characters i.e. (plant height, number of nodules and dry weight of nodules/10 plants) at all sampling dates and fresh and dry yield (ton)/fed. Also, quantitativetraits (Crude protein (CP %) crude fiber (CF), water soluble carbohydrate (WSC %) ether extract (EE) and natural detergent fiber (NDF %) in both seasons. Inoculation Dual (Rhizobium + A- mycorrhizal gave the highest values of growth, yield and quantitative traits of clover plants in both seasons. The effective treatments for growth characters fresh and dry yield (ton)/fed, as well as quantitative treatments were obtained from applying foliar micronutrient spraying at two times with dual inoculation (Rhizobium + A- mycorrhizal)

Keywords: Egyptian clover, micronutrients, dual inoculation, growth, yield, quality characters

INTRODUCTION

Egyptian clover (*Triflouim alexandrinum*, L.) is the most important forage crops in Egypt, it is cultivated in about 2.5 million feddan and used as animal feed and soil improvement. Forage quality is the most important character of feed staff producing and feeding the highest quality forage possibly increases animal performances reduces feeding costs and ultimately results in an increased return on time and money invested in forage production (Abdel-Sattar et al., 1996, Abdel-Halim et al., 1993 and Abdel – Gawad, 2003).

The foliar spray is more essential than soil application due to higher utilization which makes the nutrients more efficient. It can, also, be used to satisfy a cut- need of macro, micronutrients. Moreover, some soil fertilization problem can early to solve by foliar spray application. It acts as micronutrient on one hand and environmental toxic factor on the other hand and is known to affect nodulation and nitrogen fixation (Gaure et al., 2012), successful development of nodules by rhizobial species at many different stages of development (Brewin, 1991).

Rhizobial surface component play an important role in deciding the host compatibility and abrining about the infection leading to nodulation and nitrogen fixation (Swamyntan and Singh, 1995).

Mycorrhizal are multifunction organisms in agro ecosystems that improve soil physical, chemical and biological properties by developing mycelium, increasing nutrients absorption and soil nutrients (Cardoso and Kuyper, 2006). Chaicki et al. (2015), reported that dry matter of berseen clover (*triflouim alexandrinum*, L.) inoculation with mycorrhizal was significantly more

than control treatment. According to the same report co- inoculation by Rhizobium bacteria and Mycorrhizal fungi increased clove shoot dry weight and leaf area index compared by control. Therefore, the objective of the research is to study the effect of dual inoculation with rhizobium & A- mycorrhizal fungi and micronutrients on productivity of Egyptian clover.

MATERIALS AND METHODS

Two field experiments were carried out at the Experimental Farm of Faculty of Agriculture (Saba Basha), Alexandria. University, at Abees region Alexandria, Egypt, during the two growing seasons of 2014/2015 and 2015/2016 to study the effect of dual inoculation with Rhizobium & A- mycorrhizal fungi and micronutrients on productivity and quantitative traits of Egyptian clover.

The experiment was designed in as split plot with three replicates. The main plots were allocated to the calcium and micronutrient B at 100 g/fed for foliar spray (untreated, one spray and two spray), the four dual inoculation i.e. (uninoculation, Rhizobium, A-mycorrhizal and mixture Rhizobium + A- mycorrhizal) were allocated randomly to the sub plots. Analysis of chemical and physical properties of the experimental soil (0 to 30 cm) is shown in Table (1) according to methods reported by Page et al. (1982).

Table (1).The physical and chemical properties of the experimental soil during 2014/2015 and 2015/2016 seasons

Soil properties	2014/2015	2015/2016
A- Mechanical analysis		
Sand	13.90	14.80
Clay	44.00	43.00
Silt	42.10	42.70
Soil texture	Sand clay	
B- Chemical analysis		
pH (1:1)	7.80	7.90
EC (1:1) dS/m	3.40	3.45
1- Soluble cations (1:2) (cmol/kg soil)		
K ⁺	1.53	1.55
Ca ⁺⁺	1.95	1.90
Mg ⁺⁺	18.5	18.4
Na ⁺⁺	13.50	13.8
2- Soluble anions (1:2) (cmol/kg soil)		
CO ₃ ⁻ + HCO ₃ ⁻	2.90	2.80
CL ⁻	20.4	18.80
SO ₄ ⁻	12.50	12.80
Calcium carbonate (%)	7.60	7.50
Organic matter (%)	0.90	1.00
Total nitrogen (%)	0.44	0.48
Available Phosphorus (mg/kg)	10.8	11.3
Available K (mg/kg)	123.60	118.70

The plot area was 10.5 m² (1/400 feddan) and seed were broadcasted at the rate of 20 kg/fed. (Variety Giza 6). All plots received 30 kg P₂O₅/fed, prior to planting date (Oct. 10th and Oct 12th first and second seasons). Berseem seed was inoculated prior to sowing with *Rhizobium legumonsarumbiovartrifolii*. The rhizobia strains were provided by the biofertilizer production unit. Soil. water and Environmental Research institute. ARC. Vie ARC 101 (RE1) isolated from nodulated between (*Triflouim alexandrinum*, L.) root plants isolation and purification were done according to the method described by Vincent (1970). Apeat – based inoculum containing $\geq 10^8$ cell/g was used in seed inoculation.

A-mycorrhizal fungi (*Glomusm acrocarpuim*) strain was obtained from Department of Plant Production, Faculty of Agriculture (Saba Basha), Alexandria. University, at the rate of 2550 spores was mixed with seeds and decating technique as described by Radwan (1996) three cuts were taken through the growing period of both seasons. Cutting was done when the stand of plots was about 40- 50 cm height and the stubble height was about 6 cm from the surface. Plant samples were taken on 45 days after sowing to determine the following parameters:

A) Growth and yield characters:

1. Plant height at three cutting.
2. Number of nodules
3. Dry weight of nodules (g/10 plants).
4. Fresh and dry yield (ton/fed).

B) Quantitative traits

Plant samples were collected from each plot at each cutting weighted dried and ground in a grinding mill to pass through a 1mm seive. Samples of each cut were analyzed for forage quality properties. Crude protein (CP), crude fiber (CF), water soluble carbohydrate (WSC%), Ether extract (EE), Ash and digetative dry matter (DOD) according to A. O. A. C. (1990) and natural detergent fiber (NDF%) collected according to Mcdonald *et al.* (1978)

Statistical analysis

The obtained data were statistically analyzed for ANOVA and LSD values were calculated to test the differences between the mean values of the studied treatments according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

A- Growth characters and yield:

The obtained results given in Tables (2, 3, 4 and 5) clearly showed that the application foliar micronutrients at two times exhibited a significant effect on growth characters and yield i.e. plant height, number of nodules, dry weight of nodules/10 plants, fresh and dry yield forage (ton)/fed in both seasons. Application foliar at two sprays of some micronutrients significantly increased the growth characters at all sampling and fresh, dry yield (ton)/fed during both seasons. These results may be due to the effect of calcium and Boron on stimulation physiological processes plant photosynthetic carbohydrate and

protein accumulation, as well as sugar translocation in plant. Similar results were reported by Mohamed and Helal (1999), Nadian (2004) and Dheri *et al.* (2007).

Data in the same Tables show the effect of dual inoculation Rhizobium & A- mycorrhizal on growth characters (plant height, number of nodules and dry weight of nodules/10 plant) at all sampling and fresh and dry yield (ton)/fed, in both seasons. Growth characters and yield were improved by the dual inoculation (Rhizobium & A-mycorrhizal) which caused significant increase in growth character and yield in both seasons.

Rhizobium + A-mycorrhizal increase the ability of host plant to uptake soluble nutrient, particularly phosphorus and some micronutrients (Shabani *et al.*, 2011). Also, Nadian *et al.* (1998) reported that dry matter of berseem clover (*Trifolium alexandrinum*, L.) inoculated with mycorrhizal was significantly more than control treatment.

The interaction between application of some micronutrients and dual inoculation was significant for growth characters (plant height, number of nodules and dry weight of nodules/10 plant) at all sampling and fresh and dry yields (ton)/fed in both seasons. Tables (2, 3, 4 and 5).

Table (2). Plant height (cm) as affected by micronutrients and dual inoculation (Rhizobium, Mycorrhizal) at three cuts of *T. alexandrinum* during 2014/2015 and 2015/2016 seasons

Treatments	2014/2015			2015/2016		
	Days after sowing			Days after sowing		
	Cut1	Cut2	Cut3	Cut1	Cut2	Cut3
A) Micronutrients						
Control	30.71c	32.12c	33.17c	26.79c	27.79c	29.65c
One spray	32.44b	33.94b	35.96b	28.13b	29.75b	31.74b
Two spray	34.12a	35.48a	36.99a	30.46a	32.53a	34.65a
L.S.D. (0.05)	0.85	1.00	1.00	0.90	1.02	1.05
B) Dual inoculation						
Uninoculation	23.87d	25.03d	26.07d	23.57d	25.73c	26.97
Rhizobium	32.75c	33.83c	35.71c	25.77c	27.47	29.53e
Mycorrhiza	34.09b	36.67b	38.46b	30.46b	32.13b	34.18b
Dual (Rhiz + Mycor)	38.18a	39.84a	41.23a	34.02a	35.36a	37.37a
L.S.D. (0.05)	1.20	1.25	1.30	1.05	1.30	1.40
Interactions						
AxB	*	*	*	*	*	*

Mean values in the same column marked with the same letter are not significantly differed at 0.05 levels of probability

* significant at 0.05 level of probability

Table (3). Number of nodules as affected by micronutrients and dual inoculation (Rhizobium, Mycorrhizal) at three cuts during 2014/2015 and 2015/2016 seasons

Treatments	2014/2015			2015/2016		
	Cut1	Cut2	Cut3	Cut1	Cut2	Cut3
A) Micronutrients						
Control	113.75c	115.71	118.18c	1296.85c	128.38c	129.25c
One spray	122.59b	124.67b	126.63b	134.42b	136.00b	137.25b
Two spray	133.67a	135.75a	137.20a	144.99a	146.53a	147.88a
L.O.S.D. (0.05)	3.50	3.70	3.90	4.20	4.40	4.40
B) Dual inoculation						
Uninoculation	110.67d	112.83d	115.20d	125.44d	126.63d	128.07d
Rhizobium	126.89b	128.93b	130.50b	137.44b	139.03b	139.67b
Mycorrhiza	117.11c	119.11c	121.17c	130.66c	132.33c	133.83c
Dual (Rhiz + Mycor)	138.67a	140.72a	142.63a	148.17a	149.89a	150.77a
L.S.D. (0.05)	3.70	4.00	4.20	3.80	3.90	4.20
Interactions						
AxB	*	*	*	*	*	*

Mean values in the same column marked with the same letter are not significantly differed

At 0.05 levels of probability

* Significant at 0.05 level of probability

Table (4). Fresh and dry yield (ton)/fed as affected by micronutrients and dual inoculation (Rhizobium, Mycorrhizal) during 2014/2015 and 2015/2016 seasons

Treatments	Fresh yield 2014/2015		Dry yield 2015/2016	
	2014/2015	2015/2016	2014/2015	2015/2016
A) Micronutrients				
Control	36.33c	33.58c	3.89c	3.79c
One spray	42.30b	36.25b	4.91b	4.67b
Two spray	44.40a	41.88	5.19a	4.98a
L.O.S.D. (0.05)	1.70	1.90	0.20	0.25
B) Dual inoculation				
Uninoculation	33.42d	31.06d	3.25d	3.37d
Rhizobium	39.91c	34.89c	4.27c	3.94c
Mycorrhiza	43.89b	40.00b	5.19b	4.56b
Dual (Rhiz + Mycor)	47.32a	43.00a	5.96	5.25a
L.S.D. (0.05)	2.10	2.50	0.25	0.30
Interactions				
AxB	*	*	*	*

Mean values in the same column marked with the same letter are not significantly differed at 0.05 levels of probability

* Significant at 0.05 level of probability

Table (5). Dry weight of nodules (g/10 plants) as affected by micronutrients and dual inoculation (Rhizobium, Mycorrhizal) at three cuts during 2014/2015 and 2015/2016 seasons

Treatments	2014/2015			2015/2016		
	Cut1	Cut2	Cut3	Cut1	Cut2	Cut3
A) Micronutrients						
Control	108.75c	110.38c	112.28c	121.75	123.13c	125.00c
One spray	116.33b	118.25b	120.25b	128.92	130.75b	132.75b
Two spray	128.09a	129.25a	130.88a	139.58	141.25a	143.25a
L.O.S.D. (0.05)	3.50	3.70	3.80	3.80	3.90	4.20
B) Dual inoculation						
Uninoculation	104.00d	105.83d	107.86d	120.76d	122.33d	124.33d
Rhizobium	122.55b	123.83b	125.33b	131.44b	133.33b	135.33b
Mycorrhiza	110.89c	112.33c	114.50c	125.33c	128.83c	128.67c
Dual (Rhiz + Mycor)	133.45a	135.17a	136.83a	142.78a	144.33a	146.33a
L.S.D. (0.05)	3.30	3.50	3.60	3.50	3.70	3.90
Interactions						
AxB	*	*	*	*	*	*

Mean values in the same column marked with the same letter are not significantly differed at 0.05 levels of probability

* Significant at 0.05 level of probability

B- Qualitative traits or quality:

The results recorded in Tables (6 and 7) showed that quantitative traits i.e. crude protein (CF %), crude fiber (CF%), water soluble carbohydrate (WSC%), ether extract (EE), ASH%, natural detergent fiber (NDF%) and Degeative dry matter (DMD%) for the two seasons, were significantly affected by some micronutrients. Foliar application at two spraying significantly increased qualitative traits in both seasons. It could be concluded that the using micronutrients led to active indol acetic acid and then this acids makes amino acids to qualitative traits through this clover quality increase and by using micro and macronutrients, dry yield by of clover plant in will increased. Similar results were reported by Ali *et al.* (2012), Bhat (2013) and Bhatte *et al.* (2016).

Chemical constituents, crude protein (CF), crude fiber (CF), Ether Extract (EE), water soluble carbohydrate (WSC %), ASH%, natural detergent fiber (NDF %) and Digestive dry matter (DMD %) (Yield (ton)/fed) area shown in Table (6). Dual inoculation (Rhizobium + A-mycorrhizal) gave highest values of chemical constituents with compared to uninoculation (control) treatment in both seasons. This results could be explained by beneficial effects of fertilizer inoculation (Rhizobium + A-mycorrhizal) which led to increase nutrient supply, improve photosynthesis and ultimately provide the better qualitative characters (Gholamhosiane *et al.*, 2012). Similar results were reported by Zeidi *et al.* (2004), Canbolat *et al.* (2006), Blaise *et al.* (2006) and Abo Taleb *et al.* (2008).

The interaction between application of micronutrients and dual inoculation was significant for quantitative traits during both seasons Table (6). The highest values of quantitative traits were recorded for application of micronutrients at spray with dual inoculation (Rhizobium + A-mycorrhizal) in both seasons.

It was concluded that dual inoculation with (Rhizobium + A-mycorrhizal) increased growth, yield quantity and quality of Egyptian clover (Giza 6). The dual inoculation led to significant decrease in production cost and guaranteed more beneficial effects on social and environmental health.

Table (6a).Crude protein (TCP %), Crude fiber (CF %),Total soluble carbohydrate (WSC %) Ether extract (%), ASH (%), Natural detergent fiber (NDF %) and Degradable dry matter (DMD %) as affected by micronutrients and dual inoculation (Rhizobium, Mycorrhizal) in 2014/2015 season

Treatments	Crude protein (TCP %)	Crude fiber (CF %)	Total soluble carbohydrate (WSC %)	Ether extract (%)	ASH%	NDF%	DMD%
	2014/2015	2014/2015	2014/2015	2014/2015	2014/2015	2014/2015	2014/2015
A) Micronutrients							
Control	19.58c	24.67c	10.00c	14.33c	7.76c	47.33c	44.05c
One spray	22.17b	26.88b	10.42b	16.50b	8.26b	44.67b	47.17b
Two spray	23.63a	28.42a	11.00a	17.80a	8.44a	53.00a	49.66a
L.S.D. (0.05)	0.80	1.60	0.35	0.90	0.15	1.90	2.10
B) Dual inoculation							
Uninoculation	18.89d	25.00d	9.78d	14.78c	7.50d	46.55d	44.06d
Rhizobium	21.21c	26.00c	10.22c	15.89bc	8.09c	49.33c	46.67c
Mycorrhiza	23.12b	27.44b	10.78b	17.00ab	8.35b	51.33b	48.22b
Dual (Rhiz + Mycor)	23.95a	28.17a	11.11a	17.78a	8.62a	52.78a	48.89a
L.S.D. (0.05)	0.70	0.60	0.30	1.65	0.20	1.30	0.52
Interactions							
AxB	*	*	*	*	*		*

Mean values in the same column marked with the same letter are not significantly differed at 0.05 levels of probability

* significant at 0.05 level of probability

Table (6b).Crude protein (TCP %), Crude fiber (CF %), Total soluble carbohydrate (WSC %) Ether extract (%), ASH (%), Natural detergent fiber (NDF %) Degeative dry matter (DMD %) as affected by micronutrients and dual inoculation (Rhizobuim, Mycorrhizal) in 2015/2016 season

Treatments	Crude protein (TCP %)	Crude fiber (CF %)	Total soluble carbohydrate (WSC %)	Ether extract (%)	ASH%	NDF%	DMD%
	2015/2016	2015/2016	2015/2016	2015/2016	2015/2016	2015/2016	2015/2016
A) Micronutrients							
Control	19.85c	25.09c	10.21c	14.50c	7.03c	45.75c	44.83c
One spray	22.64b	27.00b	10.48b	15.67b	7.48b	48.25b	46.75b
Two spray	23.93a	28.92a	11.20a	17.09a	7.87a	51.50a	48.75a
L.S.D. (0.05)	0.90	1.70	0.22	0.80	0.20	2.00	1.50
B) Dual inoculation							
Uninoculation	19.19d	25.49d	9.82d	14.11d	7.06d	44.89d	44.33d
Rhizobium	21.75c	26.56c	10.44c	15.33c	7.38c	47.44c	46.33c
Mycorrhiza	23.54b	27.56b	10.89b	16.33b	7.60b	49.56b	47.55b
Dual (Rhiz + Mycor)	24.10a	28.44a	11.50a	17.22a	7.81a	51.78a	48.75
L.S.D. (0.05)	0.45	0.80	0.23	0.70	0.19	1.75	1.20
Interations							
AxB	*	*	*	*	*		*

Mean values in the same column marked with the same letter are not significantly differed at 0.05 levels of probability

* significant at 0.05 level of probability

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الملخص العربي

تأثير التلقيح المزدوج الرايزوبيم وفطر الميكوريزا والعناصر الصغرى على الإنتاجية للبرسيم المصري

فتحي إبراهيم رضوان ، محمود عبد العزيز جمعة ، عصام اسماعيل قنديل ، محمود الحجاجي موسى
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أجريت تجربتان حقليتان بمزرعة كلية الزراعة (سابا باشا) - جامعة الإسكندرية - مصر خلال موسمي الزراعة ٢٠١٤/٢٠١٥، ٢٠١٥/٢٠١٦ لدراسة تأثير التلقيح المزدوج الرايزوبيم وفطر الميكوريزا والعناصر الصغرى على إنتاجية وجودة البرسيم المصري صنف (ح٦).

ويمكن تلخيص أهم النتائج فيما يلي:

إضافة (اليورون + الكالسيوم) مرتين رشاً أعطى أعلى تأثير معنوي للصفات الخضرية مثل طول النبات، عدد العقد، الوزن الجاف للعقد/١٠ نباتات) عند جميع نباتات العينات ومحصول الطازج ومحصول الوزن الجاف (طن)/فدان وأيضاً صفات الجودة والنسبة المئوية للبروتين الخام الألياف الخام، والكربوهيدرات الذائبة مستخلص الاثير، والرماد. أدى التلقيح المزدوج (الرايزوبيم + الميكوريزا) للحصول على أعلى قيم صفات المجموع الخضري والمحصول والجودة للنباتات البرسيم في كلا الموسمين. المعاملة المؤثرة على صفات المجموع الخضري والمحصول الطازج والجاف (طن)/فدان وأيضاً صفات الجودة تم الحصول عليها من الإضافة رشاً بخليط الكالسيوم واليورون مرتين مع التلقيح المزدوج الرايزوبيم + الميكوريزا.