Response of Canola (*Brassica napus* L.) to Biofertilizers under Egyptian conditions in newly reclaimed soil

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Abstract- The study was conducted in the farm of Faculty of Agriculture, Demo, Fayoum, Egypt. The soil was of loamy- sand type. During 2006/2007 and 2007/2008 season, using a split plot arrangement in randomized complete block design with three replicates, the work was done to determine the effect of adding biofertilizers (*Azotobacter + Azospirillum,* free nitrogen fixers and *Trichoderma* a phosphate solubilizing fungi) in combinations with mineral N fertilizer with either recommended dose (N_1) or half of this dose (N_2) on yield quantity and quality of two canola lines (L_6 and H_2). The results revealed that the two canola lines were significantly different in plant height, number of branches, number of pods and seed yield /plant. L_6 surpassed H_2 line for previous traits, in addition to insignificantly higher values of the other traits; T_{10}, T_{11} and T_{12} showed superiority and high productivity with saving half of the mineral N recommended dose. For interaction; H_2T_{10}, was the best in all traits except for plant height, protein and oil percentages. H_2T_4, L_6T_6 and L_6T_8, for all traits except for seed index and oil percentage. The highest productivity recoded by H_2T_{10} (N_2+*Trichoderma +Azotobacter*), H_2T_{11} (N_2 + *Azotobacter*+ *Azospirllum*) and L_6T_8 (N_2+ *Azotobacter*) which outyielded their corresponding control by 39.3, 31.8 and 23.0% a result of using only 50% of the recommended dose of N-fertilizer (N_2). The latter combination was also superior in protein content (26.5%). These three combinations could be recommended as alternative method for canola fertilization, where they had economic and safely advantages.
Keywords: *Canola, Lines, Yield*, *Azotobacter, Azospirillum* and *Trichoderma*

Introduction

Canola (*Brassica napus* L.) is one of the main oil crops in many countries especially in Canada, European Union and USA. Cultivation of canola in Egypt may provide an opportunity to overcome some of the local deficit of vegetable edible oil production, particularly it could be successfully grown during winter season in newly reclaimed land outside the old one of Nile valley to get-arround the competition with other crops occupied the old cultivated area. (Kandil, 1984; Sharaan, 1986; Ghallab and Sharaan (2002) and Sharaan *et al*., 2002). Suitability of growing canola under Egyptian conditions, compared with other oil crops, may be ascribed to its tolerance to harsh environmental influences frequently prevailing in such newly reclaimed soil such as salinity and drought (Weiss, 1983). Oilseed rape has a relatively high requirement of nitrogen where the content of this nutrient in seeds and plant tissues is greater than in most grain crops. Research on N efficiency in oilseed rape was initiated by Grami and La Croix (1977) in Canada. Since Canola is considered as a nitrogen demanding crop, the addition of 60kg/feddan nitrogen under Egyptian conditions increased its yield (Kandil, 1984). However, the higher application of mineral nitrogen fertilizers may lead to environmental pollution especially to groundwater, and soil acidification as well as increased denitrification resulting in higher emission of N_2O to the atmosphere which may impact global warming. So, the need to find alternatives was crucial. Soil microorganisms, viz. *Azotobacter* and *Azospirillum* as N_2-fixing bacteria could be a beneficial source to enhance plant growth and producing considerable amounts of biologically active substances that can promote growth of reproductive organs and increase its productivity (Sharma *et al.*1997; Khalid *et al.* 2004; Rodriguez *et al.* 2004; Ebrahimi *et al.* 2007, Yasari *et al.* 2008 and Yasari *et al.* 2009). Not much experimental work has been conducted on the use of such N_2-fixing and phosphate solubilizing microorganisms bacteria on the growth and yield of Canola. The only attempt made on canola refer to the application of inoculation with *Penicillum bilaji, Bacillus thuringiensis* and phosphate solubilizing *Rhizobacteria* for the P-uptake, vegetative growth and grain yield of Canola was performed by Freitas *et al.* (1997). Significant and positive effect on plant height and yield of Brassica plant after using *Azotobacter* was recorded by Gupta and Samnotra (2004) & Prasad and Prasad (2004). Finding alternative application for reducing the harmful effect of mineral N-fertilizers with maintaining the high yield production for canola especially in newly reclaimed soil which is considered poor in nutrients' content and microorganisms became the must. The aim of this study was directed to investigate the effect of *Azotobacter* and *Azospirillum* as free-living nitrogen fixers and *Trichoderma* as phosphate solubilizing fungi in combinations with mineral N fertilizer on the productivity of some canola lines in the newly reclaimed soil.

Materials and Methods

The experiment has been conducted in two successive seasons the farm of the Faculty of Agriculture, Demo Research Station, Fayoum University, Egypt. The soil was of loamy-sand type, with pH 7.43, EC (dsm^{-1}) 8.55, Ca^{++} 0.91, Mg^{++} 0.45, Na^+ 1.76, K^+ 0.04, HCO_3 0.07, Cl^-

1.72, SO_4 1.25, $CaCO_3$ 12.72%, organic matter 0.54% and Total N 0.25%. The study aimed to answer the question; to what extent the yield quantity and quality of the canola lines affected by combination between mineral and biofertilizers under the conditions of newly reclaimed soil?. During the field preparation, 15kg/feddan superphosphate (15.5% P_2O_5) were added. Potassium sulphate (48.5% K_2O at the rate of 50kg/feddan was added immediately before the 1st irrigation. The cultivation was carried out on 20/11/2006 and 22/11/2007 seasons, respectively using a split-plot arrangement in randomized complete block design, with three replications. The main plots contained canola lines and the fertilizer combinations were in the sub-plots. Each replicate contained 26 plots, and the plot area was 10.5m^2. Inoculated seeds of each treatment (T) were planted in hills, 10cm apart within rows of 30cm distance between them. Other cultural practices recommended for growing canola were followed.

Seeds: The seeds of the two used lines of Canola (*Brassica napus* L.) were kindly provided from Agronomy Dept., Fac .Agric, Fayoum, Egypt (Breeding Program, Ghallab and Sharaan; 2002). The two lines were; line 6 (L_6), a selected line (Drakkar variety, Germany) and H_2 (selected line originated to crossing between Canola104 and Hanna, Egypt).

Isolation of free nitrogen fixers (FNF) and phosphate dissolving fungi:

Soil samples were collected and subjected to serial dilutions on Day and Dobereiner (1975) medium; modified Ashby medium (Hegazi *et al.*1980) and Bunt and Rovira (1955) medium for the isolation of *Azospirillum, Azotobacter* and *Trichoderma,* respectively.

Prepration of Inocula:

After growth, colonies were purified and the isolates were identified to the genus level. FNF were grown on the same isolation medium but in liquid form and spore suspension for *Trichoderma* was prepared on Tween-80 broth. The isolates were left on shaking till each ml will contain 10^7-10^8cfu g^{-1} and each ml of Tween broth contained 10^4 spore/ml. The seeds were mixed with equal amounts of the suspensions in addition to 10% Arabic gum to confirm the attachment of cells and spores on seeds surface, spread and air-dried before planting.

Fertilizers' combinations: Both the recommended dose of the N-fertilizer, 60 N units/feddan(N_1) where the feddan=0.42 hectare and half of this dose (N_2) were used in combination with the biofertilizers (12 combinations + control) as follows:

- Recommended dose of nitrogen + *Trichoderma* = N_1+Tri.(T_1)
- Recommended dose of nitrogen + *Azotobacter* = N_1+Azot.(T_2)
- Recommended dose of nitrogen + *Azospirillum* = N_1+Azosp.(T_3)
- Recommended dose of nitrogen + *Trichoderma+ Azotobacter* = N_1+Tri.+Azot.(T_4)
- Recommended dose of nitrogen + *Azotobacter + Azospirillum* = N_1+Azot.+Azosp.(T_5)
- Recommended dose of nitrogen + *Trichoderma+Azotobacter + Azospirillum* = N_1+Tri.+ Azot.+Azosp. (T_6)
- Half recommended dose of nitrogen + *Trichoderma* = N_2+Tri.(T_7)
- Half recommended dose of nitrogen + *Azotobacter* = N_2+Azot.(T_8)
- Half recommended dose of nitrogen + *Azospirillum* = N_2+Azosp.(T_9)
- Half recommended dose of nitrogen + *Trichoderma + Azotobacter* = N_2+Tri.+Azot.(T_{10})
- Half recommended dose of nitrogen + *Azotobacter + Azospirillum*= N_2+Azot.+Azosp.(T_{11})
- Half recommended dose of nitrogen + *Trichoderma+ Azotobacter + Azospirillum* = N_2+Tri.+ Azot.+Azosp. (T_{12})
- Recommended dose of nitrogen = Control (T_{13})

The seeds were treated with bacteria and fungi before planting. The nitrogen was added in three quantities the first at planting, the second at the 1st irrigation and the third at the 2nd irrigation.

At harvesting time a sample of 10 plants were taken from each plot to measure the averages of plant height (cm), number of branches/plant, number of pods and seed yield/plant. Seed index and seed yield/feddan(kg) were determined on plot basis. Seed oil and protein percentages were measured following Nuclear Magnetic Resonance (NMR) and Micro Kjeldahl digestion using automated colorimetric analysis, respectively. Combined data for the two seasons were statistically analyzed following the analysis of variance technique (ANOVA) and then the mean differences were adjudged by Duncan's Multiple Range Test (Gomez and Gomez, 1984).

Results and Discussion

Effect of lines

Results of combined data drawn in Fig.(1) show that the two canola lines were significantly different in plant height, number of branches, number of pods and seed yield /plant. L_6 surpassed H_2 line for the previous traits, in addition to it possessed insignificantly increases in the other traits. These results revealed that the well-developed L_6 line may be taken as an evidence for its high response to nitrogen and biofertilizer compared to H_2 line. In this concern, Ebrahimi *et al.* (2007) reported that rapeseed varieties had different significant effects on number of branches, but non-significant effect on protein percentage. In addition, Yasari *et al.* (2008) reported that treatment canola with mineral and biofertilizer resulted in maximum seed yield coinciding with maximum number of pods/plant.

Effect of applied different fertilizers' combinations

Data presented in Table (1) reveal that all yield and quality traits were significantly different due to different fertilizers' combinations. Treatments; T_{12}, T_8, T_{11} and T_6 for plant height (cm); 136.2, 135.5, 135.0 and 135.0, respectively were of height similar to that of control treatment. In this respect, similar results were previously recorded by Prasad and Prasad (2004). Whereas, T_3 produced the shortest plant. T_{10}, T_4, T_{11} and T_{12} for number of branches; 7.2, 6.9, 6.8 and 6.7, respectively, surpassed the control and other treatments. T_6, T_{10}, T_8 and T_4 produced higher number of pods /plant; 356, 355.2, 340 and 313.6, respectively, than those of control and most of other treatments. T_4, T_3, T_{10} followed by T_{11} and T_2 gave heavier seed index (g); 3.15, 3.14, 3.09, 2.98 and 2.96, respectively, than those of control and most of other treatments. In regard to seed yield/plant, although T_4 and T_6 which revealed full N dose possessed the highest values (28.23 & 27.55 g), T_{10} and T_8 which received half N dose produced comparable yields (26.81&26.05 g). The superiority of these four treatments due to their highest number of pods/plant. T_{12} had also similar yield (24.42 g). These results reflected the important effect of biofertilizers even with application of half N dose. Consequently, T_6, T_4, T_{10}, T_{12} and T_{11} for seed yield /fed (kg); 1273.0, 1260.0, 1237.0, 1223.0 and 1158.0, respectively, due to its recognition in yield /plant were significantly high and surpassed control (T_{13}) and other treatments. The results are in agreement with the findings of Gupta and Samnotra (2004) who concluded that simultaneous application of *Azotobacter* had a significant effect on yield of *Brassica*. T_8, T_7, T_6 followed by T_5 and T_4 were recognize in protein content (%); 26.27, 25.92, 25.90, 25.38 and 25.17. While treatments T_{12} andT_{11} for oil content (%); 44.30 and 44.0 were high but insignificantly different from control. Superiority of the above mentioned treatments, especially T_{10}, T_{11} andT_{12} (received half amount of mineral nitrogen), were desirable owing to their relative advantages, little cost of fertilization and reduced pollution in newly reclaimed soils. Similar results were previously recorded by Yasari *et al.* (2008). In a conclusion T_6 was superior in all studied characteristics except for number of branches/plant, seed index and oil percentage; while T_4 surpassed all treatments in all traits except for plant height and oil percentage. It is interesting to note that, T_{10} showed superiority for all characteristics except for plant height and yield quality. Both T_{11} and T_{12} were superior in all studied characteristics except for seed index and protein percentage.

A. Effect of interaction between lines and fertilizers' combinations

Data presented in Table (2) show that, for plant height, L_6T_{12}, H_2T_{13}, L_6T_{13}, L_6T_1, L_6T_6, L_6T_8, L_6T_{11}, L_6T_7, H_2T_8, H_2T_4 and H_2T_{11} had tallest plants; 141.2, 138.2, 137.8, 137.8, 137.2, 136.8, 135.8, 134.8, 134.2, 134.2 and 134.2, respectively; H_2T_{10}(8.0), L_6T_2(7.8), L_6T_6(7.6), H_2T_4(7.4), followed by L_6T_{12}(7.0), H_2T_{11}(6.9) and L_6T_9(6.9) gave the greatest number of branches/plant. H_2T_{10}(466.0), L_6T_8(438.0) possessed the highest number of pods/plant followed by L_6T_6(381.2) and H_2T_4(340.2); concerning seed yield /plant, L_6T_8 (35.9), H_2T_{10}(35.3), H_2T_4(33.25) and L_6T_6(32.29) produced the largest weights; H_2T_3, L_6T_4, H_2T_{10}, followed by H_2T_9 and H_2T_{11} gave the heaviest seed index (g); 3.32, 3.27, 3.24, 3.1 and 3.08, respectively. In regard to seed yield /feddan, L_6T_1(1338.0) and L_6T_6(1327.0kg), as well as H_2T_4(1327.0), H_2T_{10}(1393.0) and H_2T_{11}(1318.0 kg) produced the highest yields, followed by L_6T_2 (1296.0), L_6T_8(1239.0), H_2T_{12}(1226.0), L_6T_{12} (1219.0), H_2T_6(1218.0) and L_6T_4(1194.0 kg). These results revealed that again the importance of biofertilizers either with L_6 and H_2 lines. L_6T_8, L_6T_7, H_2T_6, H_2T_8 followed by, L_6T_5, H_2T_5, H_2T_7, L_6T_6, L_6T_4, H_2T_4, H_2T_9, L_6T_9 and L_6T_{11} gave the highest protein content percentage; 26.53, 26.50, 26.50, 26.02, 25.38, 25.38, 25.33, 25.30, 25.22, 25.12, 25.03, 24.88 and 24.78, respectively and L_6T_{13}, H_2T_{12}, L_6T_{12}, followed by L_6T_4, H_2T_{13}, L_6T_{11}, H_2T_{11}, L_6T_3 and H_2T_3 gave the highest oil percentage; 44.40, 44.40, 44.20, 44.07, 44.00, 44.00, 44.00, 43.97 and 43.92, respectively. The results are in agreement with the findings of Grami and La Croix (1977) and Yasari *et al.*(2008) in their study on canola; Suneja and Lakshminaraya (2001) on Indian mustard; Ozturk *et al.* (2003) on barley; Singh *et al.* (2005) on sorghum and Cecilia *et al.* (2004) on wheat. It could be concluded that H_2T_{10}, was the best in all studied traits except for plant height, protein and oil percentages; H_2T_4, L_6T_6 and L_6T_8 for all studied traits except for seed index and oil percentage. It's worth mentioning that the highest productivity of H_2T_{10} (N_2+*Trichoderma +Azotobacter*), H_2T_{11} (N_2+ *Azotobacter+Azospirillum*) and L_6T_8 (N_2+ *Azotobacter*) was a result of using only 50% of the recommended dose of nitrogen fertilizer which in turn is an economic advantage.

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Traits

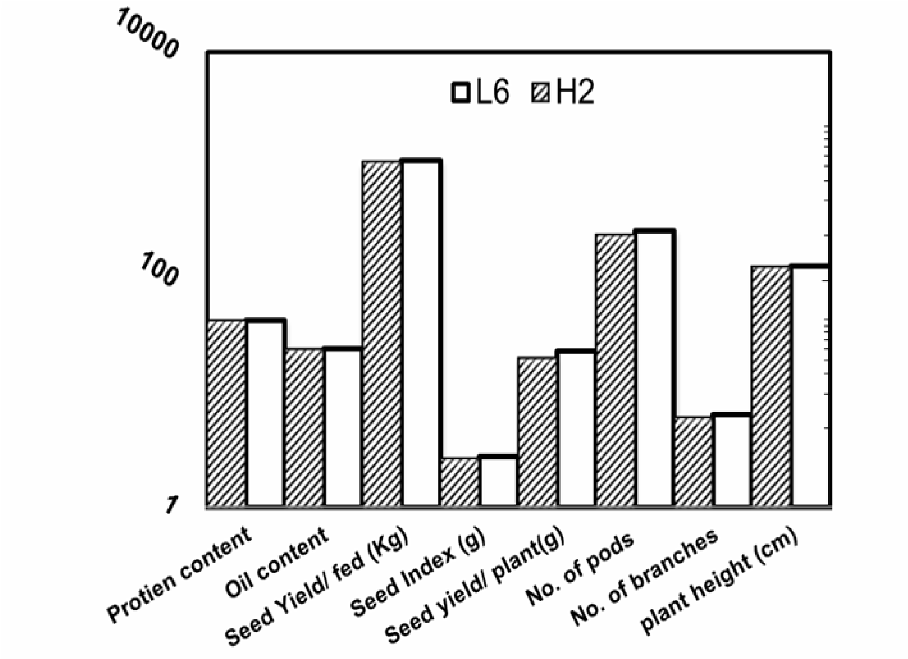

Fig. 1- Effect of lines on yield, its components and quality traits (over two seasons).

Table 1- Effect of applied different fertilizers' combinations on canola seed yield, yield components and quality traits over the two seasons.

fertilizers' combinations	plant height (cm)	No. of branches	No. of pods	Seed yield/ plant(g)	Seed Index (g)	Seed Yield/ fed (Kg)	Oil content	Protein content
N_1+Tri.(T_1)	131.4bc	6.050def	202.3c	21.77bc	2.620fg	1147.0abc	43.75d	24.36ef
N_1+Azot.(T_2)	127.2cd	6.600bc	244.1bc	20.09c	2.967bc	1073.0cde	43.77d	24.31ef
N_1+Azosp.(T_3)	121.6 e	5.950ef	197.7c	14.96d	3.138a	899.0f	43.94bcd	24.25 f
N_1+Tri.+Azot.(T_4)	131.5bc	6.850ab	313.6a	28.23a	3.148a	1260.0ab	43.98bc	25.17bc
N_1+Azot.+Azosp.(T_5)	128.5cd	5.700f	197.9c	18.79c	2.784de	935.1ef	43.83bcd	25.38b
N_1+Tri.+ Azot.+Azosp. (T_6)	135.0ab	6.550bcd	356.0a	27.55a	2.469h	1273.0a	43.55e	25.90a
N_2+Tri.(T_7)	128.8cd	6.200cdef	197.8c	21.25bc	2.253i	950.7ef	43.15f	25.92 a
N_2+Azot.(T_8)	135.5ab	6.500bcd	340.0a	26.05a	2.709ef	1112.0bcd	43.06fg	26.27a
N_2+Azosp.(T_9)	125.6de	6.400bcde	240.0bc	21.23bc	2.911cd	953.9ef	42.95g	24.96cd
N_2+Tri.+Azot.(T_{10})	125.7de	7.200a	355.2a	26.81a	3.086ab	1237.0ab	43.79cd	24.58 def
N_2+Azot.+Azosp.(T_{11})	135.0ab	6.800ab	209.5bc	21.44bc	2.983bc	1158.0abc	44.00ab	24.68de
N_2+Tri.+ Azot.+Azosp. (T_{12})	136.2ab	6.700abc	252.0b	24.42ab	2.626fg	1223.0ab	44.30a	24.54ef
Control (T_{13})	138.0a	5.900ef	225.4bc	12.91d	2.557gh	1001.0def	44.20a	24.41ef

Mean followed by the same letter(s) in each column are not significantly different (Duncan multiple range test 5%)

Table 2- Effect of interaction between lines and different fertilizers' combinations on canola seed yield, yield components and quality traits over the two seasons.

Lines X fertilizers' combinations	plant height (cm)	No. of branches	No. of pods	Seed yield/ plant(g)	Seed Index (g)	Seed Yield/ fed (Kg)	Oil content	Protein content
L_6 x T_1 (N_1+Tri.)	137.8ab	6.300defg	228.0fghi	28.52bc	2.790ghijk	1338.0a	43.70def	24.68defghi
L_6 x T_2 (N_1+Azot.)	133.2bcd	7.800 a	320.8cd	26.10cde	3.007defg	1269.0ab	43.70def	23.90k
L_6 x T_3 (N_1+Azosp.)	123.2fgh	5.900ghi	186.0hi	13.50kl	2.957defgh	946.7f	43.97bcd	24.12ijk
L_6 x T_4 (N_1+Tri.+Azot.)	128.8cdefg	6.300defg	287.0def	23.22cdefg	3.270ab	1194.0abcde	44.07bc	25.22bcde
L_6 x T_5 (N_1+Azot.+Azosp.)	129.0cdefg	5.700ghi	209.0ghi	17.00hijk	2.842fghij	941.2f	43.87cde	25.38b
L6 x T_6 (N_1+Tri.+ Azot.+Azosp.)	137.2ab	7.600ab	381.2bc	32.12ab	2.607klm	1327.0a	43.63efg	25.30bcd
L_6 x T_7 (N_2+Tri.)	134.8abcd	6.500defg	227.8fghi	24.17cdef	2.270o	976.3ef	43.40g	26.50a
L_6 x T_8 (N_2+Azot.)	136.8ab	6.200efgh	438.0ab	35.90a	2.852efghij	1239.0abc	42.72i	26.53a
L_6 x T_9 (N_2+Azosp.)	131.2bcde	6.900bcde	208.7ghi	17.60ghijk	2.720ijkl	866.2f	42.90 hi	24.88bcdefg
L_6 x T_{10} (N_2+Tri.+Azot.)	122.2gh	6.400defg	244.3efgh	18.32ghijk	2.932defghi	1081.0bcdef	43.70def	24.53fghij
L_6 x T_{11} (N_2+Azot.+Azosp.)	135.8abc	6.700cdef	222.0fghi	23.08cdefg	2.887defghij	998.7def	44.00bcd	24.78bcdefgh
L_6 x T_{12} (N_2+Tri.+ Azot.+Azosp.)	141.2a	7.000bcd	302.0de	26.53cd	2.732hijkl	1219.0abcd	44.20ab	24.70defghi
L_6 x T_{13} (control)	137.8ab	5.800ghi	238.2efghi	15.43ijkl	2.427mno	1002.0ef	44.40a	24.18hijk
H_2 x T_1 (N_1+Tri.)	125.0efgh	5.800ghi	176.7hi	15.03ijkl	2.450mno	956.7f	43.80cde	24.03jk
H_2 x T_2 (N_1+Azot.)	121.2h	5.400i	167.3i	14.08jkl	2.927defghi	877.2f	43.83cde	24.72defghi
H_2 x T_3 (N_1+Azosp.)	120.0h	6.000fghi	209.3ghi	16.42ijk	3.320a	851.3f	43.92bcde	24.38ghijk
H_2 x T_4 (N_1+Tri.+Azot.)	134.2abcd	7.400abc	340.2cd	33.23ab	3.027cdef	1327.0 a	43.90cde	25.12bcdef
H_2 x T_5 (N_1+Azot.+Azosp.)	128.0defg	5.700ghi	186.9hi	20.58efghi	2.727hijkl	929.0f	43.80cde	25.38b
H_2 x T_6 (N_1+Tri.+ Azot.+Azosp.)	132.8bcd	5.500hi	330.8cd	22.98cdefg	2.332no	1218.0abcd	43.47fg	26.50a
H_2 x T_7 (N_2+Tri.)	122.8fgh	5.900ghi	167.8i	18.33ghijk	2.237o	925.0f	42.90 hi	25.30bc
H_2 x T_8 (N_2+Azot.)	134.2abcd	6.800cde	242.0efghi	16.19ijk	2.567klm	984.0ef	43.40g	26.02a
H_2 x T_9 (N_2+Azosp.)	120.0h	5.900ghi	271.3defg	24.87cdef	3.102bcd	1042.0cdef	43.00h	25.03bcdef
H_2 x T_{10} (N_2+Tri.+Azot.)	129.2cdef	8.000 a	466.0a	35.30a	3.240abc	1393.0 a	43.88cde	24.62efghij
H_2 x T_{11} (N_2+Azot.+Azosp.)	134.2abcd	6.900bcde	197.0hi	19.80fghij	3.080bcde	1318.0 a	44.00bcd	24.58fghij
H_2 x T_{12} (N_2+Tri.+ Azot.+Azosp.)	131.2bcde	6.400defg	202.0ghi	22.32defgh	2.520lmn	1226.0abc	44.40 a	24.38ghijk
H_2 x T_{13} (control)	138.2ab	6.000fghi	212.7ghi	10.39l	2.687jkl	1000.0ef	44.00bcd	24.63efghij

Mean followed by the same letter(s) in each column are not significantly different (Duncan multiple range test 5%)