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Using rapid field test methods to assess the Potato canopy nitrogen status in the presence of organic and inorganic nitrogen fertilizer

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Abstract

Nitrogen management is one of the most important factors in potato production. Petiole nitrate concentration and SPAD reading are the most commonly used methods to assess the plant nitrogen status in cultivation period. In the present study on field experiment, the effects of the use of nitrogen fertilizer (65, 130 and 260 kg ha^{-1}), green manure (fallow and Perko), dairy cattle manure (0 and 25 ton ha^{-1}), and their combination was evaluated on the petiole nitrate concentration and SPAD reading, from 20^{th} to the 76^{th} day after the emergence (DAE), by using the rapid field test method in 2012 with the help of Randomized Complete Block Design (RCBD) with the factorial-split arrangement of four experimental replications in Iran. Total tuber yield was also determined at the end of the growing season. The results revealed that the administration of nitrogen fertilizer and green manure had positive effects on the SPAD reading and petiole nitrate concentration. SPAD readings showed a significant positive change in the presence of dairy cattle manure at the 48^{th} to 76^{th} DAE. At the 76^{th} DAE, the effects of the combination of cattle and green manure were significant on the petiole nitrate. While, the most significant and highest concentration of petiole nitrate was obtained after the administration of green manure + zero cattle manure. Nitrogen fertilizer, green manure and the combination of green and cattle manure had significant effects on the tuber yield. In this experiment, maximum tuber yield (27.185 tons ha^{-1}) was obtained by utilizing green manure + zero cattle manure.

Keywords : Petiole nitrate, SPAD, tuber yield, green manure, Dairy cattle manure and mineral fertilizer

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Introduction

The potato has an effective role in human nutrition and is an important crop worldwide (Bártová *et al.*, 2012). Tuber production involves various elements and amongst them, the nitrogen plays a vital role in the better and higher yield of potatoes (Bélanger *et al.*, 2000). The soil nitrogen deficiency can lead to decreased potato yield. On the other hand, its excessive use can cause delayed tuber growth and have a negative effect on the environment and tuber quality (Blumenthal *et al.*, 2008; Goffart *et al.*, 2008, Najm *et al.*, 2010; Najm *et al.*, 2013).

The importance and vital need of nitrogen for better plant growth and the negative effects of chemical fertilizers have generated interest in the use of organic nitrogen sources (Ashwini and Sridhar, 2006; Najm *et al.*, 2012). Animal and green manure are two important sources of organic fertilizer, which contain considerable amount of nitrogen (Laegreid *et al.*, 1999).

The cattle manure is being used from centuries to increase the soil fertility. It is a rich source of nutrients (especially, nitrogen), and can make a valuable contribution to soil's the organic matter. It can also improve the chemical, physical and biological characteristics of the soil (Kolay, 2007; Tagoe *et al.*, 2008; Benke *et al.*, 2009; Mir and Quadri, 2009).

Green manure is a crop biomass, plowed into the soil to supply plant elements (especially, nitrogen), and provides adequate amounts of organic matter to the soil. Catch crop is a quick-growing crop and its fast growth is owed to the use of green manure. The catch crop must retain more soil nitrates and undergo decreased nitrate leaching (Gowariker *et al.*, 2009). *Brassica* crops which are used as a catch crop in potato rotations, is associated with the mechanism of reduced nitrate leaching.

Because of the critical role of nitrogen administration on the plant growth and yield, estimating the plant nitrogen-status during growth season is necessary. Petiole nitrate concentration and leaf chlorophyll content are the most routinely used methods to assess the plant nitrogen status in the growth season (Zebarth *et al.*, 2012).

Hand held tools for plant analysis are considered to allow the accurate, quick and clear assessment of crop nitrogen status in the farms (Gianquinto *et al.*, 2004). Nitrate content in petiole sap can be measured by different tools which, in general, are highly correlated with the results of conventional laboratory analyses. An ion-specific electrode, as Horiba-Cardy Meter is one of the fastest methods, which directly measures the nitrate concentration (Ziadi *et al.*, 2012). Aguilera *et al.* (2012) reported that the ion meter has a significant correlation with leaf's total nitrogen content and it might prove to be an effective instrument of assessing the soil's nitrogen concentration in potato farms (Aguilera *et al.*, 2014).

The chlorophyll content of different crops can be estimated with a rapid and effective method, using the SPAD-502 meter. This method was first explored in Japan. It calculates leaf nitrogen status by estimating the light transmittance through the leaves. This device is easy-to-use and does not require any sample preparation. Many researchers have reported that SPAD reading is correlated with laboratory measurements of chlorophyll content and nitrogen concentration in leaves (Vos and Bom, 1993; Agostini *et al.*, 2010).

The purpose of the present study was to determine the changes in SPAD reading, petiole nitrate during the growth season and potato tuber yield at the harvest time in Agria potato, by the application of synthetic nitrogen fertilizer, green manure, dairy cattle manure and their combination in Iran.

Materials and Methods

Study design and experimental area

The field experiment was conducted in March 2012 at the Agricultural Experiment Station of Islamic Azad University, Karaj Branch, Alborz, Iran. The study field lies between 35° 43' N altitude, 50° 39' E longitude and altitude of 1170 m above the sea level. The experimental soil was clay loam with 0.37% organic carbon and the pH of 8.15. The P_2O_5 (available), K_2O (available), total nitrogen, Mn, Fe, Zn and Cu contents were 36.88 ppm, 353.506 ppm, 0.0168%, 2 mg kg^{-1}, 0.3mg kg^{-1}, 0.555mg kg^{-1} and 0.528 mg kg^{-1}, respectively. Mean annual precipitation in this region was estimated around 247.3 mm per year. The mean annual temperature was 14.4°C, with a maximum temperature of 42°C and a minimum temperature of -20°C.

The experiment was laid out in the factorial-split plot arrangement, based on Randomized Complete Block Design (RCBD) with four replications. The treatment groups were administered with the nitrogen fertilizer of urea source in three levels (65, 130 and 260 Kg nitrogen ha^{-1}), green manure (fallow and Perko), and dairy cattle manure (0 and 25 tons ha^{-1}).

Seeding and fertilizer administration

Green manure (Perko) was added to the field on 13th March, 2012 and was plowed by rototiller on 21st

May, 2012. Dairy cattle manure (1-year-old compost) was analyzed before application and was applied before tuber plantation (Table-1). Nitrogen fertilizer was applied during two phases of tuber cultivation: planting and growing phase. Potatoes of Agria variety were planted in the field on 3rd June, 2012. The plot size was considered to be 15 m2. Tuber seeds were hand-planted in the furrows of four rows with the depth of 15 cm, inter-row spacing of 75 cm and the intra-row spacing of 25 cm. The irrigation was through dripper irrigation system.

Estimation of SPAD, petiole nitrate level and the tuber yield

From the 20th to 76th day after emergence (DAE), the leaf SPAD level and petiole nitrate amount were measured from youngest fully expanded leaves (4th leaf from the top) at the interval of every 15 days. The SPAD value was observed in the field by handheld chlorophyll meter (Hansa Tech Chlorophyll content Meter, Model Cl-01). Six readings were made for the leaflets of the youngest fully expanded leaves of each of the six plants in each plot (Zebarth *et al.*, 2012).

For estimating the nitrate concentration of petiole, the samples were collected in each plot with all the leaflets removed, and the petioles were put into paper bags (to avoid exposure to the light), and placed into a cooler. The samples were transported to the lab, and then separately analyzed for nitrate content (Vos and Bom, 1993; Zebarth *et al.*, 2007; Aguilera *et al.*, 2014).

Petiole nitrate was measured by handheld nitrate meter (Compact No^{3-} Meter; B-343, twin No3-, Horiba Ltd., Japan). According to instruction manual of the device, the nitrate meter was initially calibrated before analysis by two nitrate standards (150 and 2000 ppm Standard solution of No^{3-}, Horiba Ltd.) and alternatively checked during the analysis (Anonymous, 2008; Aguilera *et al.*, 2014). Samples were milled (6 samples for each plot), several drops of the resultant sap were placed on the sensor with a clinical dropper and the petiole nitrate concentration was measured in ppm (Aguilera *et al.*, 2014).

Tubers were harvested on October 25, 2012 in a 2 m length of the two middle rows of experimental plot, excluding 0.5 m from each end of the plots. After harvesting, the total yield was determined for each plot and reported as ton ha^{-1}.

Statistical analyses

Analysis of Variance (ANOVA) was performed using the General Linear Model (GLM) procedure of SAS (SAS Institute, 2010; Version: 9.3, SAS Institute Inc. Cary, NC, USA). Graphs and charts for nitrogen fertilizer, green manure and dairy cattle manure were obtained by using Prism 5 for Windows (Graph Pad Software, 2007; Graph Pad Software Inc. San Diego, CA). For the determination of the interaction among treatment groups, the mean values were compared using Duncan's Multiple Range Test (DMRT). The results were considered to be significant at the probability level of $P < 0.05$.

Table - 1. Some properties of the manure samples, used in the present study.

Parameters	Cattle manure
Organic carbon (%)	26
C: N (%)	40.47
Total Nitrogen (%)	0.6424
Total elemental content ($mg\ kg^{-1}$)	
P	317.7
K	41026.5
Fe	1700
Mn	270
Cu	45
Zn	172

Results and Discussion

Petiole nitrate

Table 2 shows that the nitrogen fertilizer had a significant effect on the petiole nitrate concentration at all sampling times. During all experiments, the petiole nitrate content was relatively low in the presence of reduced concentration of nitrogen fertilizer, but reached to a maximum at higher concentrations of nitrogen fertilizer (Fig.-1). These results are in agreement with the reports of previous researches (Li *et al.*, 2010; Zebarth *et al.*, 2012). Vos and Bom (1993) have reported that higher values of petiole nitrate were obtained at the maximum experimental concentration of nitrogen fertilizer and the low petiole content vice versa. This phenomenon shows that the petiole's nitrogen content is directly related to the nitrogen amount administered.

During the present experiment, the highest amounts of petiole nitrate concentration were estimated on 34th DAE in all the treatment groups, followed by the administration of 65, 130 and 260 Kg N ha^{-1}: 3726. 719, 4973.438, and 5962.500 ppm, respectively. On the other hand, in all treatment groups, the lowest amounts of petiole nitrate content were obtained on 76th DAE (1968.750, 2162.500000, and 2837.50000 ppm respectively; Fig.-1). These results are in line with report of Love *et al.* (2005), that the petiole nitrate content was affected by both plant age and the level of nitrogen fertilizer administered to the crop (Love *et al.*, 2005).

Petiole nitrate content showed considerable changes after the application of green manure during all experimental groups (Table - 2). Figure 2 shows that, however, on 20th DAE, petiole nitrate content showed minor difference within the fallow and green manure, in other times there were significant differences between this two treatments. Perko is a catch crop and the main goal of planting a catch crop is to retain the adequate level of nitrates in the soil that might be lost by leaching. So, it can be expected that, Perko treatment may increase the petiole nitrate level (Gregorova *et al.*, 1993; Gowariker *et al.*, 2009). Highest amounts of nitrate content in both treatment groups (fallow and green manure group) were achieved on 34th DAE, which were estimated to be 4543.750 and 5231.350 ppm, respectively, whereas, the lowest amounts were obtained on 76th DAE (Fig.-2).

At the 76th DAE, the combinational administration of cattle and green manure showed significant results (Table - 2). Figure 3 is related to the DMRT analysis of the manure-administered field results. The results showed that the highest concentration of petiole nitrate content was obtained when the green manure was administered alone ($P< 0.05$), which may be because of the beneficial effects of green manure. Nasri *et al.* (2014) reported that the pre-planting of Perko before the cultivation of wheat can cause the increased level of the nitrogen harvest index than the administration of fallow to the experimental plots (Nasri *et al.*, 2014a).

Whereas, the lowest concentration of petiole nitrate content was recorded from fallow cattle manure. There were no significant differences, recorded in DMRT, between this treatment and fallow + cattle manure. The DMRT did not show any significant differences between the effects of fallow + cattle manure and green manure + cattle manure combinations. The cattle manure + fallow and cattle manure + green manure had positive effects on the petiole nitrate level, but these effects were not impressively significant (Fig.-3). It is thought that the above-described phenomenon depends on the nitrogen and carbon quantity of organic manure and C/N balance in the soil, which can subsequently affect the plant nitrogen component (Abreu *et al.*, 2007). It was observed in the

Table - 2. Analysis of variance for SPAD readings, petiole nitrate content and Tuber yields.

Source of variation	Df	Day After Emergence (DAE)										Tuber Yield
		20th		34th		48th		62nd		76th		
		SPAD	Petiole Nitrate	SPAD	Petiole Nitrate	SPAD	Petiole Nitrate	SPAD	Petiole Nitrate	SPAD	Petiole Nitrate	
Rep.	3	14.783 ns	184745 ns	47.125**	495278.60 ns	4.162 ns	3676173.19**	35.992**	1954538.57**	0.946 ns	30208.333 ns	87.687*
Nitrogen (N)	2	87.338**	11135456.87**	190.356**	20083386.85**	124.908**	21763494.19**	110.037**	10050069.17**	163.977**	3327708.333**	167.994*
Green Manure (G)	11	37.701**	1596419.56*	93.437*	5673593.88**	76.962**	8123833.52*	61.880*	5590528.16*	99.360**	5950208.333**	518.17**
N × G	2	5.524 ns	548615.05 ns	3.8537 ns	65324.35 ns	7.617 ns	3344104.77 ns	2.009 ns	177979.33 ns	15.627 ns	101458.333 ns	39.313 ns
Error	15	4.293	279780.11	16.323	494260.89	6.205	1366954.82	7.619	1119457.85	2.540	283375	27.328
Cattle Manure (C)	1	8.944 ns	192691.70 ns	8.2917 ns	471537.63 ns	37.418*	753253.52 ns	28.799*	222598.47 ns	35.604**	630208.333 ns	3.537 ns
N × C	2	16.194 ns	111179.37 ns	4.034 ns	119510.29 ns	3.4361ns	366057.27 ns	0.5146 ns	175113.12 ns	3.005 ns	65208.333 ns	49.601 ns
G × C	1	0.806 ns	12683.13 ns	2.336 ns	15318.88 ns	0.3201 ns	2989509.19 ns	0.026 ns	119375.81 ns	3.090 ns	3466875.000**	153.904*
N × G × C	2	15.498 ns	142277.80 ns	0.9128 ns	323010.29 ns	4.796 ns	1799276.69 ns	1.230 ns	31021.32 ns	1.371 ns	625.000 ns	63.654 ns
Error	18	7.941	307352.07	7.7904	399766.97	5.811	694438.4	3.767	373923.34	3.99375	167013.89	25.459
CV		12.530	17.37919	10.892	12.93636	11.745	18.19671	10.967	19.06338	12.337	17.59311	22.54574

**P<0.01; *P<0.05; ns = not significant

present study that the cattle manure observed to contain low amounts of nitrogen and high amount of C/N balance. The result is also revealing that the effects of treatment of experimental crop with cattle manure on the petiole nitrate were comparatively lower (Table – 2 and Fig.-3).

SPAD analysis

The present study showed that the nitrogen content of the added fertilizer had positively significant effects on the SPAD reading in all experiments (Table - 2). Figure 4 shows that the highest SPAD reading in all the experimental plots were obtained at the concentration of 260 kg N ha^{-1} and the lowest amounts were achieved after the administration of 65 kg N ha^{-1}. While, the administration of 130 kg N ha^{-1} resulted in a relatively medium range of SPAD value. Nitrogen shortage hinders the chlorophyll formation and causes a sharp decline in the leaf chlorophyll content. So, it is widely accepted that the Chlorophyll content is affected by the concentration of nitrogen fertilizer added to the crop (Westermann, 2005; Martín *et al.*, 2007; Najm *et al.*, 2012).

During the early growth season, the SPAD reading showed minor differences among the treatment groups, containing different concentrations of nitrogen, but these differences were intensified during the period of 34th to 48th DAE. Whereas, during the period of 62nd to 76th DAE, only 260 kg N ha^{-1} showed significant effects on the SPAD reading, as compared to other treatment groups. While, the administration of 65 and 130 kg N ha^{-1} had a comparatively lesser effect on the SPAD value (Fig.- 4). These results are in line with the report of Vos and Bom (1993).

The maximum amounts of SPAD reading in all the treatment groups were obtained on 34th DAE, following the administration of 65, 130 and 260 Kg

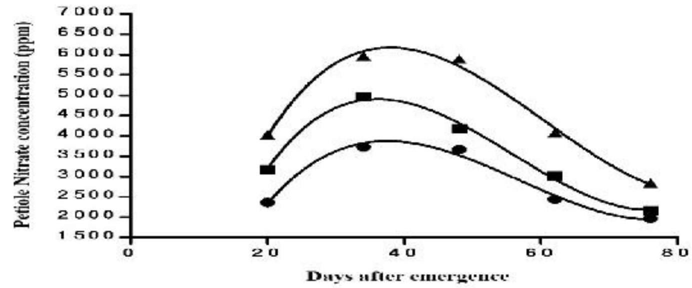

Fig.- 1. Petiole nitrate concentration as a function of time and nitrogen fertilizer concentration (65 Kg Nitrogen ha^{-1} ●; 130 Kg Nitrogen ha^{-1} ■; and 260 Kg Nitrogen ha^{-1} ▲).

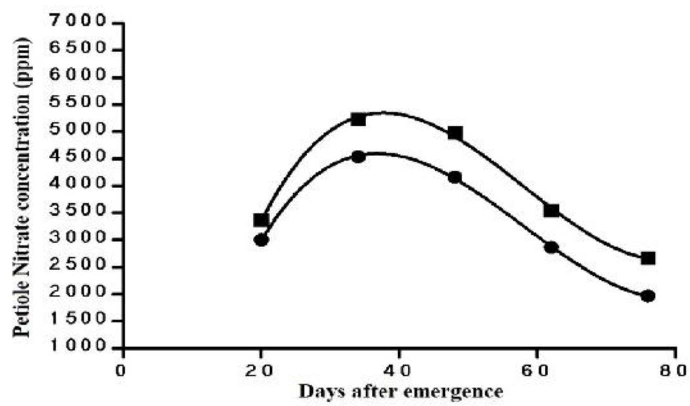

Fig.-2. Petiole nitrate concentrationas a function of time and green manure (fallow●; green manure (perko)■).

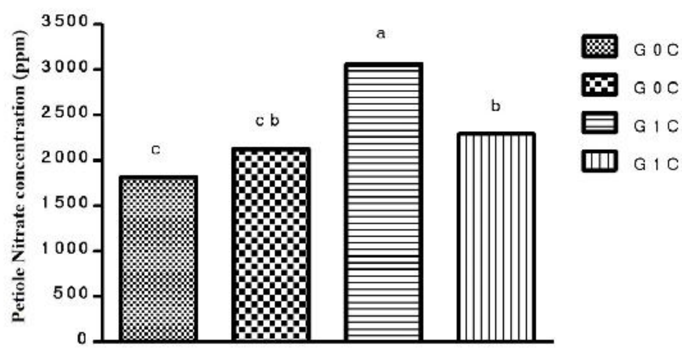

Fig.-3. The effect of combination of cattle manure and green manure on petiole nitrate content at the 76th DAE (G0C0: fallow+ zero cattle manure, G0C1:fallow+ 25 ton cattle manure ha^{-1}, G1C0: Green manure+ zero cattle manure, and G1C1: Green manure + 25 ton cattle manure ha^{-1}). Values with the same letter are not significantly different (*P* >0.05).

N ha^{-1}: 22.47, 25.08, and 29.30, respectively (Fig.-4). Also, in all treatment groups, minimum ranges of SPAD were achieved on 76th DAE (13.90, 14.82, and 19.85625, respectively). It corroborates with the finding of Mauromicale *et al.* (2006) that, chlorophyll content was increased with increasing nitrogen level, and was decreased with increasing plant age.

Table 2 shows that the Perko, as green manure, had impressively significant effects on the SPAD value. During all the sampling periods, the SPAD readings reached highest amount, after the addition of green manure. The maximum amounts of SPAD readings in both treatment groups (fallow and green manure; 24.23 and 27.02, respectively) were obtained on 34th DAE. Whereas, the lowest amounts (14.76 and 17.64, respectively) were achieved on 76th DAE (Fig.-5).

Chlorophyll formation can be affected by nitrogen and other elements. Perko, as a green manure catch crop, can store sufficient amounts of nitrogen and other elements by absorbing them and transferring to the later crops. Perko can play a major role in increasing the chlorophyll content by supplying a fraction of the vital nutrients (Gowariker *et al.*, 2009; Ghorbani *et al.*, 2010).

SPAD readings in the present study, showed significant changes in the presence of cattle manure at the 48th, 62nd and 76th DAE. Figure 6 shows that, although, during early growth season, the SPAD readings had minor difference within the 0 and 25 ton cattle manure ha^{-1}, it increased over the period of 48th to 76th DAE, and the highest SPAD reading were obtained in the treatment crops of which maximum was administered with 25 ton cattle manure/ha.

Chlorophyll synthesis can be affected by changing the magnesium, iron, calcium, manganese and zinc contents (Martín *et al.*, 2007; Najm *et al.*, 2012). Cattle manure, used in the present experiment, was rich in the

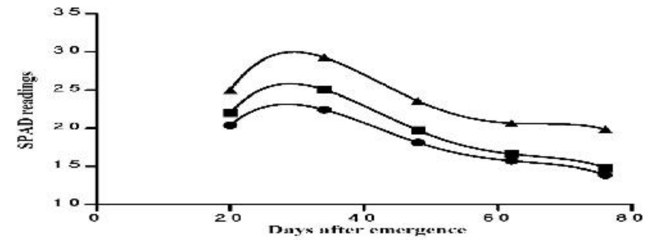

Fig.-4. SPAD readings as a function of time and nitrogen fertilizer concentration (65 Kg Nitrogen ha^{-1} ●; 130 Kg Nitrogen ha^{-1} ■; and 260 Kg Nitrogen ha^{-1} ▲).

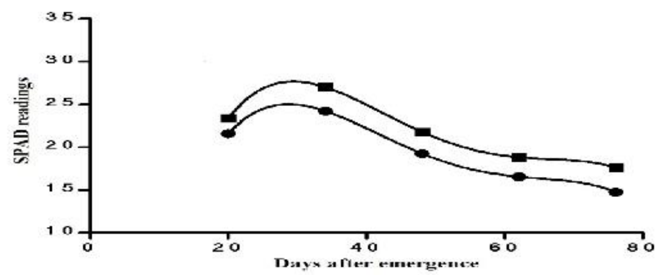

Fig.-5. SPAD readings as a function of time and green manure (fallow●; green manure(perko)■).

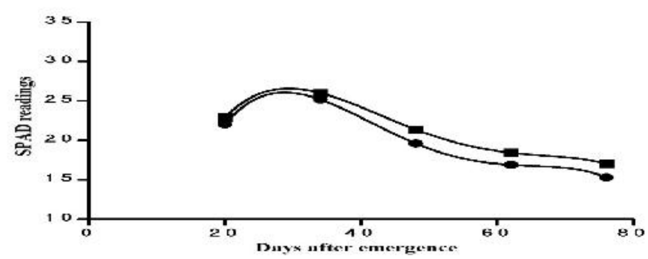

Fig.- 6. SPAD readings as a function of time and cattle manure (zero cattle manure●; 25 ton cattle manure ha^{-1} ■).

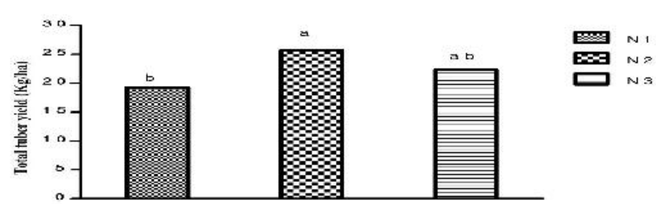

Fig. -7. The effect of nitrogen fertilizer on the potato tuber yield (N1:65 kg nitrogen ha^{-1}, N2: 130 kg nitrogen ha^{-1}, and N3 260 kgnitrogen ha^{-1}). Values with the same letter are not significantly different (*P* >0.05).

above-stated elements (Table-1). Consequently, manure plays a special role in increasing the chlorophyll content by supplying a fraction of these nutrients and enhancing the solubility of some necessary elements (Clemente *et al.*, 2007; Kolay, 2007). Beneficial effects of manure on chlorophyll content are also reported in other plants (Ghosh *et al.,* 2004; Bokhtiar and Sakurai, 2005).

Tuber yields

During the present analysis, the tuber yield showed significant change in the presence of nitrogen fertilizer (Table - 2). The tuber yield was relatively lower in the presence of 65 kg nitrogen ha^{-1}, but it reached to a maximum amount at 130 kg nitrogen ha^{-1}. The DMRT analysis showed significant difference between the two treatment groups ($p \leq 0.05$; Fig.-7). Tuber yields reached to the maximum amount by increasing the concentration of nitrogen fertilizer, because nitrogen improves photosynthetic capability, plant growth and subsequently the total crop yield (Zvomuya *et al.*, 2002; Kumar *et al.*, 2007; Najm *et al.*, 2012). Whereas, the tuber yield was decreased when experimental crop was administered with 260 kg nitrogen ha^{-1} (Fig.-7). The results of previous studies have shown that the application of excessive amount of nitrogen fertilizer leads to delayed maturity and an imbalance between the sink and source, and sub-sequently can reduce the tuber yield (Kumar *et al.*, 2007 ; Najm *et al.*, 2012).

Green manure alone and the combination of cattle manure and green manure had significantly positive effects on the tuber yield (Table - 2). Figure 8 shows that the maximum amount of tuber yields was obtained from the plots, administered with the green manure + zero cattle manure. While, the addition of green manure + cattle manure showed comparatively less significant effect on the tuber yield. There were no specific differences

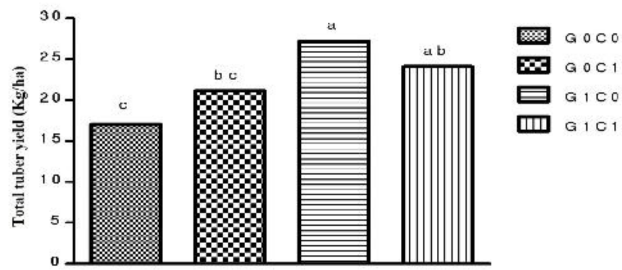

Fig.- 8.The effect of combination of cattle manure on the total tuber yield (G0C0: fallow+ zero cattle manure, G0C1: fallow+ 25 ton cattle manure ha^{-1}, G1C0 : Green manure+ zero cattle manure, and G1C1: green manure + 25 ton cattle manure ha^{-1}) . Values with the same letter are not significantly different (P >0.05).

in the tuber yield between the two treatment groups. This increase might be due to the improved chemical, physical and biological properties of the soil and increased nutrient uptake (especially the nitrogen). These factors have a positive effect on the plant growth, photosynthesis and assimilation rates, which result in better crop yield (Opena and Porter, 1999; Abou-Hussein *et al.*, 2003; Yan *et al.*, 2007; Benke *et al.*, 2009; Gowariker *et al.*, 2009).

The lowest amount of tuber yield was obtained from fallow + zero cattle manure. The DMRT analysis did not show any significant difference between this treatment group and the group, administered with cattle manure + fallow cattle manure. So the effects of treatment with cattle manure on the tuber yield were lower than green manure + fallow (Table 2 and Fig.- 8).

Conclusions

It can be concluded from the present study that the separate application of nitrogen fertilizer had a positive effect on the chlorophyll content and petiole nitrate concentration during the growth season. The highest amount of these characteristics was obtained when the crop field was added with the maximum amount of nitrogen fertilizer. Although, the nitrogen fertilizer had positive effect on tuber yield, its excessive amount decreases the tuber yield. The plant is a risk for low tubur yield , when petiole nitrate concentrations and chlorophyll content remain in the high range by the administration of excessive amount of nitrogen fertilizer. On the otherhand, when these characteristics remain in the optimal range, the crop received sufficient nitrogen to obtain high tuber yields. The present study was also revealed that the green manure had stronger effects on chlorophyll content, petiole nitrate concentration (during growth season) and tuber yield, as compared to the effect of cattle manure. The maximum amount of tuber yield was obtained by utilizing green manure + fallow. Therefore, it can be suggested that the application of perko as green manure is a suitable solution for providing sufficient nitrogen and other elements to the plant, that increasing chlorophyll concentration and subsequently achieving maximum yield.

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