Biological Forum – An International Journal **7**(2): 498-508(2015)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Influence of 3,4-Dimethylpyrazole Phosphate as a Nitrification Inhibitor on Nitrogen Dynamics in Different Wheat Varieties by the Isotopes

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(Received 18 June, 2015, Accepted 08 August, 2015)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: In order to evaluate the effects of combined application of DMPP on different wheat varieties (different Δ) a greenhouse experiment was carried out in a factorial randomized complete block design (CRD) in three replications. Factors were five wheat varieties and three fertilization systems. In order to trace nitrogen dynamics, 15N enriched ammonium sulfate was used. Results showed that genotypes with lower Δ index prefer to absorb more ammonium (instead of nitrate). The delay in the release of nitrate by DMPP, caused nitrogen fertilizer as greater source of nutrient suppliers in wheat. This leads to increase wheat yield production in DMPP treatment. We can concluded that in the region with the possibility of winter freezing, DMPP application can prevent the conversion of ammonium to nitrate and consequently the nitrogen fertilizer through the leaching decrease and apart from increasing the level of crop production, fertilizer use efficiency can be followed.

Keywords: 3, 4-Dimethylpyrazole Phosphate, 15N, Carbon isotope discrimination, Inhibitor, Nitrification, Wheat.

INTRODUCTION

Most of nitrogen fertilizers are rapidly oxidized to nitrate by microorganisms in the soil. Nitrate is highly soluble in water and due to its mobility in the soil; the potential for leaching is particularly high. The excessive use of chemical fertilizers in agricultural areas is a major cause of groundwater pollution (Adams, *et al*., 1994; ChangEntz, 1996; Fraters, *et al*., 1998; Thomsen, *et al*., 1993). Apart from environmental hazards, nitrate leaching is one of the most important ways of soil nitrogen loss (Shen, *et al*., 2003). Therefore low nitrogen fertilizer use efficiency will be a direct consequence of this process (CamargoAlonso, 2006; Li, J., *et al*., 2003). In this regard, the delay in biological oxidation of ammonia using inhibitors can reduce the loss of nitrogen through leaching process (Zerulla, *et al*., 2001). Furthermore, this process can reduce the rhizosphere pH and thus uptake of phosphorus will increase (Amberger, 1989).

Nitrification inhibitors are special compounds, which delay biological oxidation of ammonium to nitrite (without influence on oxidation of nitrite to nitrate). In other words, only the first step of nitrification (oxidation of ammonium to hydroxylamine) are influenced by inhibitors and the second step (oxidation of toxic nitrite) remains unaffected (Li, H., *et al*., 2008; Weiske, *et al*., 2001). This is takes place by preventing (or interfering) with the metabolism of bacteria involved in nitrification (such as *Nitrosomonas*) (Kiyani, 2010). In the climate with high soil nitrogen leaching potential, the positive impact of nitrification inhibitors have been reported (Yu, *et al*., 2007). Also use of nitrification inhibitors has significant effects on reducing emissions gases (such as N_2O CH_4) from soil to the atmosphere (Chen, *et al*., 2010; Malla, *et al*., 2005). In addition, the increasing nitrogen fertilizer use efficiency and yield production of crops are one of the most important advantages of applications of inhibitors (Li, J., *et al*., 2003; RocoBlu, 2006). Since several types of nitrification inhibitors are marketed.

These compounds are capable of inhibiting activity of *Nitrosomonas* bacteria. The end result of this process is making delay on biological oxidation of ammonium (Zerulla, *et al*., 2001). Thus significant reduction in the formation of nitrate ions in the soil, due to the use of inhibitors has been reported (Di, H. Cameron, K., 2005; Islam, *et al*., 2007). This was associated with a reduction in the atmosphere N_2O (Di, *et al*., 2007; Magalhaes Chalk, 1987; Merino, *et al*., 2005).

DMPP (3, 4-dimethylpyrazole phosphate) is a new nitrification inhibitor with desirable properties. Today the different types of fertilizers combined DMPP with various formulations of fertilizer available on the market and use in the field (Kiyani, 2010). DMPP along with mineral fertilizers (nitrate and ammonium) in amounts of less than a tenth of DCD used (0.50 to 1.5 kgha-1) can delay the process of nitrification for 10 weeks. Of course the amount of delay will vary, depending on climatic conditions and the characteristics of the studied locations (Barth, *et al*., 2001; Chen, *et al*., 2010; Zerulla, *et al*., 2001). It is reported that 3, 4-dimethylpyrazole phosphate can influence the quality or quantity of product and also reduces nitrate leaching in soil and water (Chen, *et al*., 2010; Li, H., *et al*., 2008) and mitigate the amount of gas emission N_2O of soil (ChangEntz, 1996). This compound is very beneficial with regard to inhibition of nitrification processes in the soil (RocoBlu, 2006; Yu, *et al*., 2007; Yu, *et al*., 2008) and in terms of toxicity tests (quality control process) no toxicological issues have been reported (Zerulla, *et al*., 2001).

Currently, most of activities on DMPP focused on effectiveness of nitrification inhibition process in the soil and improvement of yield product (Xu, *et al*., 2005; Zerulla, *et al*., 2001). There are also few studies on the effect of DMPP on reduction of nitrogen leaching salt ions in the heavy clay soil. Considering that nitrification inhibitor DMPP (in recent years) has been entered the industrial market of Iran, so its use as normal is not very large, and consequently research activities in this field are negligible. In this respect (Ehsanpour, *et al*., 2012) were studied the effect of DMPP inhibitor and phosphorus on phosphorus use efficiency of wheat yield production. They reported that the use of DMPP along with ammonium sulphate and ammonium nitrate sulfate fertilizer leads to a significant increase in available phosphorus at the end experiment. Likewise use of DMPP will significantly increase soil ammonium concentration and decrease soil nitrate content. Accordingly, they recommend 3, 4-dimethylpyrazole phosphate in combination with ammonium nitrate phosphate to increase efficiency of phosphorus and wheat yield production.

Globally in the past twenty five years, extensive research activities have been conducted on the use of different nitrification inhibitors to reduce environmental contamination (caused by the excessive use of nitrogen fertilizers) and increase their productivity. The main activity in the first decade of studies focused on the effects of inhibitors on improvement of agricultural production. In this regard, few studies were carried out on assessment of leaching of nitrogen and other nutrients in the soil by different inhibitors (Xu, *et al*., 2005; Zerulla, *et al*., 2001). (Yu, *et al*., 2008) applied DMPP on undisturbed soil columns and found that cumulative loss of nitrate reduced by 57.5%. DMPP also makes more concentrations of ammonium in the soil and therefore cumulative loss of it will be expected. Of course this depends on the soil temperature and humidity during the test (as in higher temperature, the effect of DMPP quickly reduced). For example, at a temperature higher than 30°C, DMPP makes useless within a week.

In relation to effect of DMPP in reducing the leaching of minerals, coefficient of 27%, 24%, 34% and 26% has been reported for calcium, magnesium, potassium and sodium respectively (Di, 2005). In explaining the relationship of DMPP on nitrate reduction and three mentioned cations, they found linear equation as $Y = 3+1.22X$ (which Y: concentration of 3-cation and X: concentration of nitrate). They attributed this effect to cations carriers. Thus when nitrate is being leached a number of cations also carried out with it (for making charge balance). Therefore, anything that can reduce nitrate loss, is supposed to remain mentioned cations in mutual influence too.

According to the effect of DMPP on nitrate and nitrite microorganisms in the soil, (Li, H., *et al*., 2008) concluded that during first fortieth days *Nitrosomonas* population declined but increased again and after two weeks its population is reduced. On average, use of DMPP in rice and turnip makes significant reduction on *Nitrosomonas* population by 26% and 29% respectively. However, application of DMPP was ineffective on the Nitrobacter population. Chen *et al*. compared the inhibitory of N-serve and DMPP. They reported that N-serve makes 42 days delay to nitrification process, but in comparison, the inhibitory effect of DMPP was longer and its curve was a little bit lower than for N-serve levels (Chen, *et al*., 2010). In comparison with the results of other researchers DMPP has been more effective (than DCD) in cool weather conditions. N-serve was more effective in reducing of N_2O gas emissions. But because this substance has a high vapor pressure, it cannot be mixed with solid fertilizers and therefore will be limited of use.

Macadam, *et al*., (2003) compared DMPP and DCD and reported that both inhibitors have been successful on preventive retention of soil nitrogen to ammonium and N_2O cumulative emissions was also reduced. But due to the phytotoxic effects of DCD, the production of white clover reduced. In this regard, nutrient imbalance symptoms (which lead to chlorosis and necrosis of the edge of leaves) were considered by DCD application.

(Herrmann, *et al*., 2007) using acetylene (as a nitrification inhibitor) and 15N isotope dilution technology reported that use of one kPa acetylene (C_2H_2) prior to use of nitrogen labeled fertilizers can be a full evaluation of the of nitrification inhibition and nitrogen gross rate (before 24 hours) in soil with high potential of nitrification. (Linzmeier, *et al*., 2001) were investigated N_2O gas emissions affected by DMPP inhibition on winter wheat using ammonium and nitrate 15N labeled fertilizers. They reported that the amount of N_2O gas emissions under nitrate fertilizers will be higher than ammonium fertilizers. This process has demonstrated 20 percent reduction, as a result of the inhibitor DMPP application. The results of isotope trace technique showed that 10 to 40 percent of the N_2O gas emissions were related to fertilizer nitrogen pool and 60 to 90 percent is attributed to soil nitrogen pool. Reported results also indicated that microbial nitrogen pool acted as an important source of nitrogen waste (N_2O gas emissions). They reported that use of granular fertilizers will be lower N_2O gas emissions (compare liquid fertilizers).

Generally, the scope of activities on nitrification inhibitors can be attributed to two intersecting groups of factors. The first group includes environmental variables which reduce or increase the nitrification process (such as soil moisture and temperature, salinity and alkalinity problems, the amount of organic matter, texture and pH, distribution and extent of root exudates, population of *Nitrosomonas* bacteria, etc.). The second group includes factors affecting the effectiveness of the application of DMPP (such as increasing the efficiency of nitrogen fertilizers and enzyme activities, increase production and reduce the leaching of nitrates and other minerals, etc.). For example (Yu, *et al*., 2008) were examined the effects of soil moisture and temperature to reduce nitrate leaching under DMPP or (Li, H., *et al*., 2008) were tested population of *Nitrosomonas* bacteria to increase production of rice and turnips under application of DMPP. However activities carried out during the past 25 years has covered many of the above research. One of the research program on which no activities have been reported is the impact of different winter wheat varieties (with different carbon-13 isotope discrimination index) on nitrate inhibition and increase yield production by DMPP application. Plants with lower carbon-13 isotope discrimination associated as physiological indicators which improve water use efficiency (like wide root system development). It seems delayed conversion process of ammonium to nitrate (as a result of DMPP application) can lead to greater absorption of ammonium by the plant. In this respect to balance the electrical charges, plants release protons. This makes rhizosphere more acidic and may increase nutrient availability. The direct consequence of this phenomenon is further growth of plant. Therefore, this study aimed to evaluate the effects of combined application of nitrification inhibitor of DMPP on different wheat varieties (with different carbon-13 isotope discrimination) on fertilizer nitrogen use efficiency and yield production in relation to the qualitative and quantitative assessments of production.

MATERIALS AND METHODS

The glasshouse experiment was conducted in a factorial randomized complete block design (CRD) in three replications in 2013 at Nuclear Science and Technology Research Institute (Karaj, Iran). The first factor was wheat varieties (high Δ indexes (G1), to some extent high (G2), medium (G3), to some extent low (G4) and low (G5)) and the second factor was three fertilization systems (no-fertilizer (N), labeled ^{15}N ammonium sulfate fertilizer (F) and ^{15}N fertilizers containing DMPP nitrification inhibitor (D)). Pots with a capacity of 13.6 kg of soil were used and four winter wheat crops were planted. To improve the quality of plants and deal with preliminary salinity (resulting from the use of fertilizers), seeds were grown in culture media (Cocopeat) for 8 weeks at 4°C and vernalized and after germination, seedlings were planted directly in the pots. In order to trace nitrogen element, ammonium sulfate fertilizer labeled with 4.637 atom percent enrichment ^{15}N excess was used. Nitrogen fertilizer was applied requirement as 135 $kgha^{-1}$ in one step (before planting).
Fertilizers (included ^{15}N) was dissolved in a small volume of water and spread over the pots. To select the desired wheat genotypes with different Δ indexes (Carbon-13 isotope discrimination: $\Delta = (\delta^{13}C_{air} - \delta^{13}C_{plant})/(1+ \delta^{13}C_{plant})$ which: $\delta_{sample} = 1000 [(R_{sample}/R_{VSMOW}) - 1]$), five different genotypes were selected from genetic resources of DARI (*Iranian Dry land Agricultural Research Institute)* center (Table 1). All results of the ^{13}C and ^{15}N (in the natural abundance scale), reported as delta value (δ) and in terms of standard deviations per thousand (‰) compared to standard marine carbonate fossils (PDB) and air standard.

A DMPP fertilizer was supplied from -24 (Compo Company, Germany) mixed with ammonium sulfate. After mixing Novatec Solub with labeled with 15N, was calculated as 0.54 $kgha^{-1}$. This value is in the range of acceptable manufacturer of nitrification process (0.5 to 1.5 kg ha^{-1}) (Kiyani, 2010). DMPP were applied in the form of ammonium sulfate (Novatec Solub 21) and after mixing with 15N labeled material, its concentration reached to 0.54 $kgha^{-1}$.

Table 1: Some characteristics of genotypes, based on carbon isotope discrimination and efficiency of water.

Genotype	Variety / Line	%N	$\delta^{15}N$ †	%C	C/N	$\delta^{13}C$ ‡	Δ	Class*
G1	Homa	1.94	-1.74	54.89	28.23	-11.39	3.43	B
G2	Ohadi	2.58	-1.08	59.80	23.21	-9.89	1.91	C
G3	Sabalan	2.21	-1.56	50.99	23.06	-9.29	1.30	C
G4	PYN	2.54	-2.31	62.98	24.77	-4.62	-0.55	A
G5	Zargana-6	2.03	-0.87	59.55	29.31	-7.46	-3.40	A

† Delta values (δ), representing deviations per mil (‰) from standard air, such that sample=$1000[(R_{sample}/R_{standard})-1]$, where R is the $^{15}N/^{14}N$ or $^{13}C/^{12}C$ ratio in sample and standard

‡ Delta values (δ), representing deviations per mil (‰) from standard Pee Dee Belemnite (PDB)

* Seeds classification for water use efficiency (A=more efficient)

This value is in the range of acceptable manufacturer of nitrification process (0.5 to 1.5 kg ha^{-1}) (Kiyani, 2010). Fertilizers applied based on their critical value and soil testing, and mixed with DMPP, dissolved in a certain volume of water and applied uniformly for all pots. Before testing, physical and chemical analyzes were performed on the soil as follows: Ntotal: 0.041 (%), P: 7.81 (ppm), K: 125.5 (ppm), organic carbon: 0.42 (%), pH: 7.75, EC: 0.63 (dsm^{-1}), TDS: 375.3 (mgl^{-1}), CO_3: 0.2 ($meql^{-1}$), HCO_3: 1.33 ($meql^{-1}$), calcium carbonate equivalency: 16.0 (%), Ca: 5.54 ($meql^{-1}$), Mg: 4.42 ($meql^{-1}$), SO_4: 2.88 ($meql^{-1}$), Zn: 0.80 (ppm), Mn: 2.80 (ppm), Cu: 0.40 (ppm), Cl: 1.50 ($meql^{-1}$), FC: 16.80 (%), PWP: 9.53 (%), SP: 26.77 (%) and soil texture: sandy clay loam (sand=58.67%, silt = 20.10% and clay = 21.23%),

Soil samples were taken four times (two, four and six weeks after planting and the last one at harvest period). Ammonium measurement in the soil KCl extract were down by spectrophotometry method and colorimetric reaction at 655 nm (Mousavi Shalmani, 2008). For determination of nitrate, principles absorption spectrophotometry method was used. Ultraviolet radiation (UV) wavelength of 210 nm was used. In this method two measurements were carried out (one before (by Zn coated by Cu) and one after reduction of nitrate). Using the difference between these two measurements, concentration of nitrate in the extracts was determined (Mousavi Shalmani, 2008). After harvesting, plant samples were separated into grain and straw. Isotopic ratio $^{15}N/^{14}N$ in plant samples were measured by Rittenberg method and NOI_7 emission spectrometer at the Institute of Nuclear Science and Technology in Iran (Mousavi Shalmani, *et al*., 2009; Mousavi Shalmani Khorasani, 2003). Isotopic ratio $^{15}N/^{14}N$ in soil extracts samples were measured by micro diffusion method and NOI_7 emission spectrometer (Mousavi Shalmani, 2008). $^{13}C/^{12}C$ isotope ratio and the delta carbon-13 ($\delta^{13}C$) were measured by isotope ratio mass spectrometry laboratory of the University of Adelaide (Australia). All statistical analysis was performed with the software GenStat-14.

RESULTS AND DISCUSSION

Results showed that genotypes G4 and G5 with the lowest amounts of isotopic discrimination (-0.54 and -3.40 respectively) had the maximum amount of seed, straw and grain yield production (Fig. 1). Treatment G4 with 10.89 g/pot seed and 39.90 g/pot straw yield production was allocated to the highest level of biomass production. On the other hands, G1 and G2 treatments with an average of 6.04 g/pot seed and 28.97 g/pot straw yield had the lowest amount of production and were placed in the last statistical group. In relation to thousand seed weight (TKW), treatments G4 and G5 with an average of 39.17g placed in the first group, treatments G2 and G3 with an average of 34.11g placed in the second group and finally treatment G1 with 29.76g placed in the third group. Treatments G4 and G5 had the highest total nitrogen percentage (3.85%) in seed and then treatments G1, G2 and G3 were statistically placed in three different groups respectively. Despite significant differences in seed nitrogen content, no significant differences were found in nitrogen content of straw.

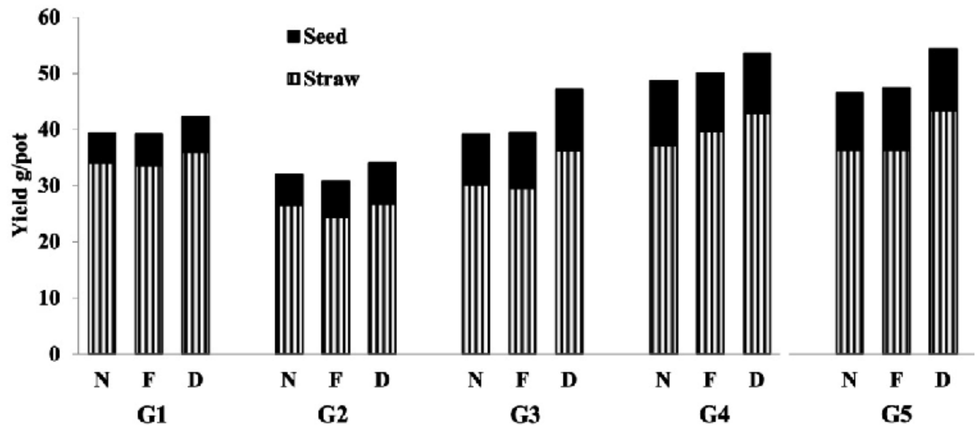

Fig. 1. Yield production (straw and seed) under different wheat genotypes and fertilization treatments (N: control, no nitrogen fertilizer, F: ^{15}N labelled ammonium sulphate, D: ^{15}N labelled ammonium sulphate with DMPP).

It seems lower ^{13}C isotope discrimination index at G4 and G5 treatments and greater efficiency in supply of nutrients, could justify increasing the percentage of nitrogen in the seed treatment. The highest amount of 15N (atom % excess) in the grain and straw were recorded in G5 treatment by 3.48% and 3.49% and the lowest level was observed in treatment G2 by 3.30% and 3.16% respectively. Therefore, treatments G4 and G5 with average 74.44% had the highest amount of nitrogen derived from labeled fertilizer (Ndff) and treatments G2 with average 71.26% were placed in the last group. Fig. 2 shows nitrogen yield derived from labeled fertilizer and soil, on different plant organs. As can be seen, most of the nitrogen in the plant derived from labeled fertilizer (than soil). This process increased by the use of DMPP. It also noted that genotypes G4, G5 and G3 absorbed more nitrogen than genotypes G1 and G2. It seems genotypes with lower isotope discrimination index could absorb higher fertilizer nutrients during filling stage of seed because of more developed root system and more extensive area for uptake of water.

The same pattern was also applicable in the case of straw. So that the maximum amount of fertilizer use efficiency were observed in treatments G5 (75.38%) and the minimum amount were observed in treatments G2 (68.28%). All mentioned processes have caused the nitrogen use efficiency of seed for treatments G1 to G4 as 10.47, 14.91, 27.28, 32.01 and 36.16 percent respectively (Fig. 3).

For straw (due to the longer growing period) values for nitrogen use efficiency in treatments G1 to G4 were recorded 29.35%, 22.90%, 23.07%, 33.17% and 38.14% respectively.

In relation to total nitrogen use efficiency in whole parts of wheat plant, treatments G4 and G5 have allocated the highest fertilizer use efficiency (69.75%) and treatments G3, G2 and G1 with values 50.34%, 39.82% and 37.81% were statistically placed in the next three groups respectively. It seems more developed and effective root system in treatments with low carbon-13 isotope discrimination index could increase nitrogen fertilizer use efficiency up to 32.35%.

Regarding the effects of nitrification inhibitors on the production of wheat seed, DMPP treatment (D) could produce 9.22 g/pot seed ($p>0.05$) and placed in the first statistical group. Then fertilizer treatments (F) and without fertilizer (N), with values of 8.66 and 8.25 g/pot seed production were statistically placed in the second and third groups. Mentioned process was also identical for the production of straw. DMPP treatment (D) could produce 46.29 g/pot of straw and placed in the first statistical group and fertilizer treatment (F) and without fertilizer treatment (N) by average 41.26 g/pot placed in the second group. Use of nitrification inhibitors had no significant effect on thousand seed weight (TKW) index and all treatments were not significant in this respect. It seems delay in release of nitrates from fertilizer have significant effect on total nitrogen concentration in the grain.

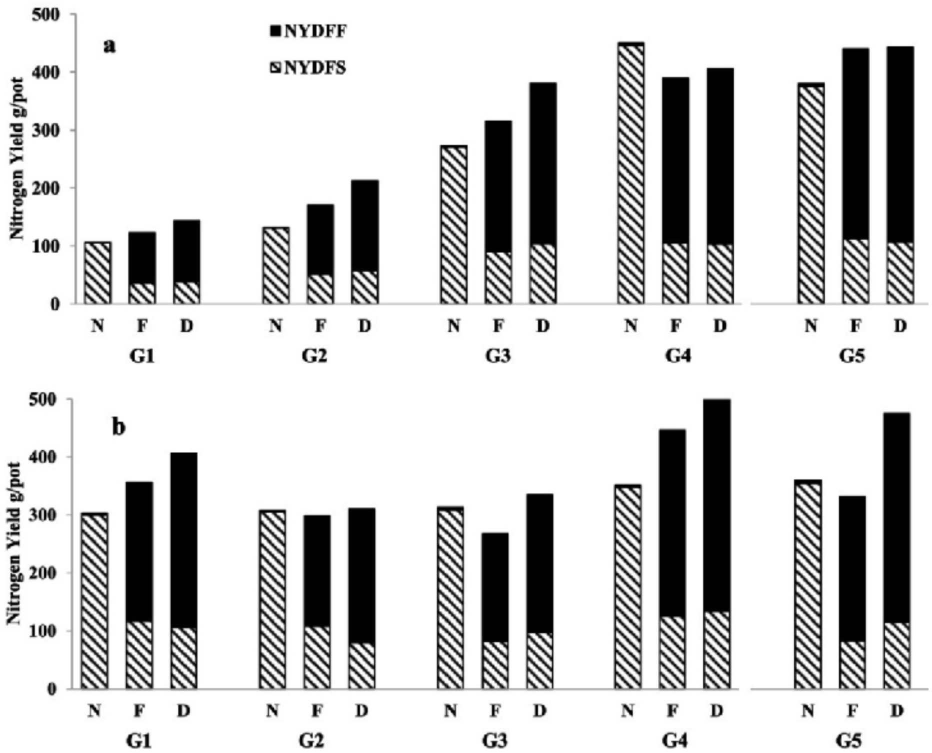

Fig. 2. Nitrogen yield derived from labeled fertilizer (NYDFF) and nitrogen yield derived from labeled soil (NYDFS) on seed (a) and straw (b) under different wheat genotypes and fertilization treatments (N: control, no nitrogen fertilizer, F: ^{15}N labelled ammonium sulphate, D: ^{15}N labelled ammonium sulphate with DMPP).

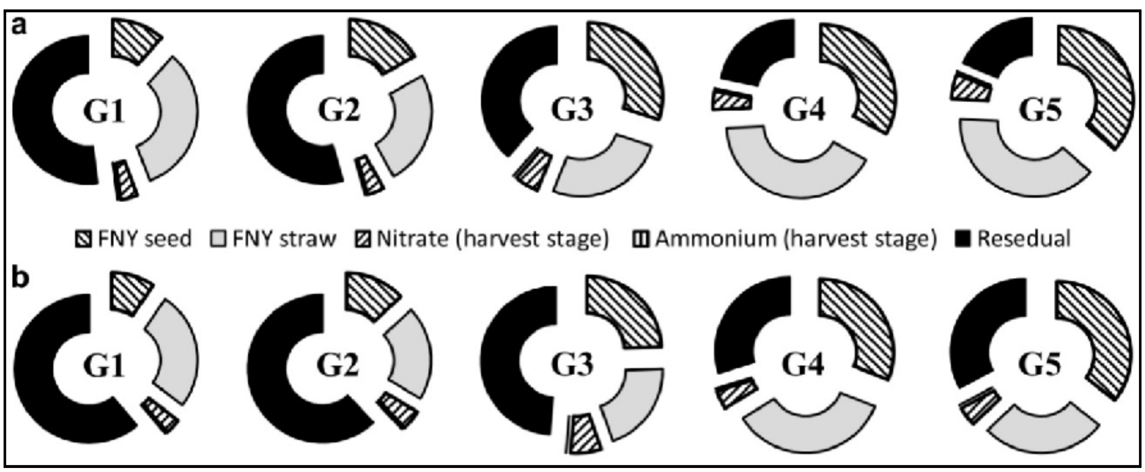

Fig. 3. Nitrogen fertilizer absorbed by different wheat genotypes and remained in to the soil under different fertilization treatments (a: ^{15}N labelled ammonium sulphate with DMPP, b: ^{15}N labelled ammonium sulphate).

Nitrification inhibitor treatment (D) with an average nitrogen content of 3.29% placed in the first group, fertilizer treatment (F) with an average of 3.16% in the second group and treatment without fertilizer (N) with an average of 3.02% in the second group.

It is worth mentioning that there is not any significant differences regard to the percentage of nitrogen in straw. In this regard the use of nitrification inhibitors has led to 73.8% of nitrogen in seeds (Ndff) derived from labeled fertilizer.

While in the condition without the use of nitrification inhibitors, 71.8% of nitrogen derived from fertilizer. In other words use of inhibitors, caused a delayed effect on nitrate release in to the soil and consequently a greater proportion of nitrogen fertilizers played a valuable rolls on wheat plant growing at filling seeds stage. The process had significant effect on straw and consequently treatments D (73.94%) and F (69.05%) were statistically placed in two different groups. Regarding the effect of nitrification inhibitors on fertilizer use efficiency in treatments D and F, these two treatments with values of 25.61% and 22.73% have been statistically placed in two different groups. Thus application of DMPP could increase 3.0% fertilizer use efficiency in wheat. In relation to the straw, it is also significant for nitrogen fertilizer use efficiency in treatments D and F which recorded as 32.8% and 25.8% respectively. In relation to the total nitrogen use efficiency (regardless of different wheat genotypes) application of DMPP inhibitor could increase nitrogen fertilizer efficiency from 31.28% (in F treatment) to 37.7% (in D treatment). Therefore, use of inhibitor in general could improve nitrogen fertilizer use efficiency by 10.0 percent.

According to the interactive effects of wheat genotype and nitrification inhibitor, the highest yield were recorded in G4 (11.50 g/pot) and the lowest were in G1 (5.27) with no application of nitrogen fertilizer (Fig. 1). The highest level of total wheat biomass were recorded in G5 treatment with application of DMPP (54.4 g/pot) and the lowest level was observed in G1 treatment with fertilizer treatment (24.4 g/pot).

It seems that the toxic effects of fertilizer treatments through the early stages of plant growth caused minimum yield of wheat. In relation to thousand seed weight (TKW), treatments F in genotype G5 and no fertilizer treatment in G4 (with an average of 40.5 g) had the highest index and no fertilizer treatment in G1 (with an average of 28.0 g) had the lowest ones.

In relation to nitrate ($N\text{-}NO_3$) and nitrate derived from labeled fertilizer ($15N\text{-}NO_3$) accumulation in the soil, the results showed that different genotypes of wheat did not show statistically significant difference. This indicate that preferred nitrate absorption in different wheat genotypes was not significant ($p>0.05$). The ammonium ($N\text{-}NH_4$) results were seen considerably different. Genotype G1 (average 81.2 mg/pot) placed in the first group, genotypes G4, G2 and G3 (average 51.5 mg/pot) placed in the second group and genotypes G5 (average 23.95 mg/pot) placed in the third group (Table 2). Analysis of ammonium derived from labeled fertilizer ($^{15}N\text{-}NH_4$) confirmed the above process. Genotype G1 (average 28.10 mg/pot) placed in the first group, genotypes G4 and G2 (average 19.78 mg/pot) placed in the second group and genotype G3 and G5 (average 9.23 mg/pot) placed in the third group.

In relation to the effect of DMPP on nitrate accumulation in the soil, the highest nitrate ($N\text{-}NO_3$) levels (87.4 mg/pot) have been resulted in the fertilizer treatment (F) and then DMPP (50.19 mg/pot) and no-fertilizer (18.98 mg/pot) treatments which were statistically placed in the second and third groups (Table 2).

Nitrogen-15 isotope tracing technique in the soil confirmed this fact that DMPP application could significantly reduce nitrate-nitrogen levels from 60.74 to 26.70 mg/pot ($p\leq0.01$). In this way, inhibition of DMPP on nitrate production re-confirmed (Fig. 1). In relation to the effect of DMPP on ammonium accumulation in the soil, the results were quite different. The N-ammonium in DMPP treatment was recorded 148.7 mg/pot and in the fertilizer treatments (F) and without fertilizer (N) with an average of 0.52 mg/pot was placed simultaneously in the second group. It can be implied, by inhibiting the conversion process of ammonium to nitrate, plenty amounts of ammonium remains in the soil. 15N tracing technique in the soil confirmed that in DMPP treatment, 36.6 percent of the ammonium nitrogen (remained in the soil) derived from labeled fertilizer. This is equivalent to 94.6 mg/pot of ammonium nitrogen derived from labeled fertilizer. In this respect, the interesting point were especial effect of DMPP in the accumulation of N-NH4 derived from labeled fertilizer and specific absorption of ammonium ions in genotypes G3 and G5 during plant growth (Table 2). It is clear that mentioned varieties could reduce the concentration of ammonium in the soil significantly. Ehdaie *et al*. reported that preferable absorption of ammonium (or nitrate) was under genetic control of wheat varieties, and the difference between genotypes can be considerable (Ehdaie, *et al*., 2006). This difference is due to the size and morphology of roots, the mineral elements required by the relative growth speed, absorption and transport of ions and efficiency of their use. Although wheat is generally preferred uptake of nitrate (compare with ammonium) (Roosta Schjoerring, 2008), but when environmental conditions is suitable for increasing the intensity of photosynthesis (for example stem elongation) and subsequent rapid growth, the absorption of ammonia is preferred and its relationship with the soil nitrate is reduced (Baligar, *et al*., 1993). Also in dryland farming areas (similar to west and northwest of the country) which cold stress is limiting factor for absorption and growth of wheat in early spring, plants prefers absorption of ammonium to nitrate.

Table 2: Changes in available nitrate and ammonium concentration in the soil, derived from soil and fertilizer in different wheat genotypes during growth period.

Genotype	Sampling duration	N-NO$_3$ mg/pot		N-NH$_4$ mg/pot	
		ANSDS*	ANSDF**	ANSDS	ANSDF
G1	after 2 weeks	4.82 ^{f} ± 0.20	43.72 ^{bcd} ± 4.09	2.51 ^{b} ± 0.34	113.95 ^{a} ± 16.21
	after 4 weeks	12.28 ^{def} ± 0.07	36.43 ^{bcd} ± 2.35	5.77 ^{b} ± 0.27	94.25 ^{a} ± 13.47
	after 6 weeks	35.44 ^{ab} ± 2.08	75.41 ^{abc} ± 7.24	59.13 ^{ab} ± 7.50	113.02 ^{a} ± 16.15
	at harvest	32.65^{abc} ± 1.94	34.93 ^{bcd} ± 0.13	85.65 ^{a} ± 11.79	0.68 ^{a} ± 0.07
G2	after 2 weeks	6.31 ^{ef} ± 0.07	47.91 ^{abcd} ± 5.23	4.84 ^{b} ± 0.34	75.09 ^{a} ± 10.72
	after 4 weeks	11.06 ^{def} ± 0.20	43.30 ^{bcd} ± 3.75	5.37 ^{b} ± 0.40	66.88 ^{a} ± 9.58
	after 6 weeks	34.55^{abc} ± 0.27	95.27 ^{a} ± 5.23	4.70 ^{b} ± 0.07	119.62 ^{a} ± 17.09
	at harvest	15.51^{cdef} ± 0.27	39.14 ^{bcd} ± 0.47	59.08 ^{ab} ± 8.17	0.47 ^{a} ± 0.07
G3	after 2 weeks	10.48 ^{def} ± 0.07	27.24 ^{cd} ± 2.95	7.24 ^{b} ± 0.60	27.07 ^{a} ± 3.89
	after 4 weeks	15.03^{cdef} ± 0.07	18.73 ^{d} ± 1.01	4.54 ^{b} ± 0.27	41.88 ^{a} ± 5.96
	after 6 weeks	32.29^{abc} ± 0.47	79.71 ^{ab} ± 2.48	5.21 ^{b} ± 0.20	74.39 ^{a} ± 10.59
	at harvest	22.34^{abcdef} ± 0.54	57.77^{abcd} ± 1.01	34.89 ^{ab} ± 4.62	0.29 ^{a} ± 0.07
G4	after 2 weeks	6.23 ^{ef} ± 0.40	28.51 ^{cd} ± 2.01	6.24 ^{b} ± 0.54	118.12 ^{a} ± 16.88
	after 4 weeks	15.95 ^{bcdef} ± 0.94	35.00^{bcd} ± 2.75	3.42 ^{b} ± 0.20	64.79 ^{a} ± 9.25
	after 6 weeks	37.38 ^{a} ± 0.74	9.816^{abcd} ±3.82	22.75 ^{ab} ± 2.68	83.00 ^{a} ± 11.86
	at harvest	28.70^{abcd} ± 0.07	5.951^{bcd} ± 0.07	61.84 ^{ab} ± 8.51	0.49 ^{a} ± 0.07
G5	after 2 weeks	14.77 ^{cdef} ± 0.07	18.07 ^{d} ± 1.47	5.38 ^{b} ± 0.27	28.12 ^{a} ± 4.02
	after 4 weeks	19.69 ^{abcdef} ± 0.20	36.52 ^{bcd} ± 3.89	6.72 ^{b} ± 0.54	29.96 ^{a} ± 4.29
	after 6 weeks	35.79 ^{ab} ± 0.87	68.01 ^{abcd} ± 4.15	9.38 ^{b} ± 0.47	49.30 ^{a} ± 7.04
	at harvest	25.38^{abcde} ± 0.13	47.68^{abcd} ± 0.87	4.23 ^{b} ± 0.20	0.04 ^{a} ± 0.07

* ANSDS: Available nitrogen in the soil, derived from soil

**ANSDF: Available nitrogen in the soil, derived from fertilizer

- The same letter in each column, refers to no significant difference at 5% level (Duncan's test).

In this respect, Breteler and Smith reported that in lower temperature, the roots of wheat (and barley) prefer ammonium absorption more than nitrate (Breteler Smith, 1974). It seems differences in the relative growth rates, morphology of the root system, absorption and translocation of ions in genotypes G3 and G5 are effective factors that preferentially absorb ammonium (more than nitrate) during plant growth.

In relation to evaluate changes in soil nitrate during growth periods, results suggest that during the sixth week the greatest amount of nitrates in the soil has been seen. After using DMPP, significant decrease in nitrate concentration has been observed (as a result of inhibition of ammonium conversion to nitrate). In relation to the ammonium, reverse situation has been seen. During initial six weeks, maximum concentration of ammonium has been detected and then decreased. It is worth mentioning that the average concentration of ammonium-N derived from labeled fertilizer at the time of harvest in fertilizer (F) and inhibitor (D) treatment were 0.04 and 0.70 mg/pot respectively. The results also indicate that the effect of DMPP (as slight increase in the concentration of ammonium) extend as well as harvest period.

The distribution pattern of labeled ammonium sulfate fertilizer in the soil and plant in different wheat genotypes under the influence of nitrification inhibitor DMPP (and without inhibitor) shows that the amount of nitrogen uptake in wheat (including seed and straw) increased with reduce carbon-13 isotope discrimination index (Δ) (Fig. 3). This indicates a significant negative correlation of wheat yield and Δ. These findings are in accordance with results of (Monneveux, *et al*., 2012) to look relationship between water use efficiency, crop production and carbon-13 isotope discrimination in wheat under mega-environments and water regimes in China and India. They reported negative and significant correlation between grain yields of hybrid wheat varieties with carbon-13 isotope discrimination index, in water stress conditions,

On the other hand (Jlibene, *et al*., 2012; Miloud, *et al*., 2012; Wahbi Shaaban, 2012) reported positive and significant correlation between wheat yield production and Δ in moisture stress conditions.

As can be seen, incompatible results in relation to negative or positive correlation between wheat yield and carbon-13 isotope discrimination index proves more research activities needed in this regard. But, based on the results of this study and negative correlation finding between wheat genotypes and 13C isotope discrimination index, it seems that the varieties with low delta index have high relative growth rates. Therefore DMPP utilization during the first months of wheat growth, leads to more relative absorption of ammonium and makes better response to yield and fertilizer. This has been clear for genotypes 3 and 4 in figure 2. In these treatments, the minimum level of nitrogen loss has been recorded and consequently increase the efficiency of fertilizer will not be unexpected.

Another interesting point in the study was increase in crop production as a result of DMPP application. As can be seen in figure 1, wheat yield production in genotypes G1, G2 and G3 are more obvious. It can be concluded that, DMPP application for the genotypes with higher carbon-13 isotope discrimination index can be enhanced with greater wheat yield product.

CONCLUSION

Statistical analysis of the data shows that genotypes with smaller 13C isotope discrimination have maximum seed and straw yield production, thousand seed weight (TKW) index, total N%, nitrogen derived from labeled fertilizer (Ndff%) and nitrogen use efficiency (NUE). It seems genotypes with lower Δ index have greater water use efficiency, due to more developed root system and the wider uptake of water. Application of DMPP increased fertilizer use efficiency in seed by three percent and in straw by seven percent (totaling 10% increase). In other words, the delay in the release of nitrate by DMPP application, caused nitrogen fertilizer as greater source of nutrient suppliers in wheat plant nutrition (in seed filling stage). This leads to increase the seeds and biological wheat yield production and total nitrogen percentage in DMPP treatment. N-nitrate concentration in the soil reduced and subsequently N-ammonium concentration increased by 11.50 mg/pot.

Regarding effectiveness of DMPP for different varieties of winter wheat, it seems certain genotypes prefer to absorb more ammonium (instead of nitrate). It is due to differences in the morphology of the root system and the absorption and transport of ions. In these genotypes and under specific conditions, use of DMPP may be increase the efficiency of nitrogen fertilizer. For instance in this trial, the use of DMPP in G5 genotype has been able to absorb about 75 percent of nitrogen derived from labeled fertilizer and consequently three-quarters of its total N-food requirement provide from fertilizer source. Regarding the effect of DMPP application on total nitrogen fertilizer use efficiency, maximum amount allocated to the G4 and G5 genotypes (with average of 75.05%). However, despite the increase of DMPP and fertilizer in genotype G1 and G2, fertilizer use efficiency is low and the amount of 43.06% was observed.

Therefore application of DMPP in the first months of wheat varieties with low delta and high relative growth rates result higher relative uptake of ammonium and this makes a better response to ammonium fertilizer. In these treatments, the minimum level of nitrogen can waste and thus increase the efficiency of fertilizer will be expected. In other words, in the region with the possibility of freezing in winter, if there is more than 45 days intervals between the planting date (of wheat) and occurrence autumn freezing, DMPP application can prevent the conversion of ammonium to nitrate and consequently the nitrogen fertilizer through the leaching decrease and apart from increasing the level of crop production, fertilizer use efficiency can be followed. It is obvious that in the areas like Iranian dry land in the North West part of country (with the possibility of cold stress as a limiting factor for plant uptake and growth in early spring), DMPP application can provide nitrogen required by the plant at stem elongation stage (with the rapid growth and photosynthesis rate) and influence the quality and quantity of wheat.

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