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# Impact of Fulvic Acid and Nitrification Inhibitor on Wheat Production and $NO_3$-N losses in Clay Soil

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TWO field experiments were conducted at Sakha Agricultural Research Station, Kafr El-Sheikh Governorate, Egypt, during two successive seasons (2017/18 and 2018/19) to study the effect of fulvic acid and nitrification inhibitor on $NO_3$ leaching and wheat productivity. The split plot design with three replicates was used with three doses of fulvic acid (i.e., 0, 4.76 and 9.52 kg ha$^{-1}$), whereas the main plots represented bynitrogen fertilizerswith and without nitrification inhibitor at rate of 90, 135 and 180 kg N ha$^{-1}$ in sub-plots. Nitrification inhibitor 3, 4-dimethylpyrazole phosphate (DMPP) was applied at rate of 8%. Results indicated that the highest mean values of soil salinity (EC), OM and available NPK were obtained from soil in plots received 180 kg N ha$^{-1}$ with DMPP + 9.52 kg fulvic acid ha$^{-1}$. The highest mean values of plant height (110.34 and 111.42 cm), 1000-grain weight (55.60 and 61.00 g), grain yield (7.62 and 7.95Mg ha$^{-1}$); straw yield (13.64 and 14.02Mg ha$^{-1}$); N concentration in grain and straw (2.09 and 0.84 %, respectively),N content of grain (152.97 and 149.56 kg N ha$^{-1}$); N content of straw (99.51 and 95.58 kg N ha$^{-1}$) and total N uptake by wheat (252.49 and 245.14 kg N ha$^{-1}$) were recorded by 180 kg N ha$^{-1}$ combined with 8% DMPP + 9.52 kg fulvic acid ha$^{-1}$ in first and second seasons, respectively. Meanwhile, the lowest leaching values in both seasons were achieved in plots received 90 kg N ha$^{-1}$combined with 8% DMPP + 9.52 kg fulvic acid ha$^{-1}$ after 4$^{th}$ irrigation. Based on the results of the current study, the combinations of fulvic acid and nitrogen fertilizer with DMPP can be considered as integrated nutrient management to improve the soil fertility and wheat productivity and save the environment from pollution.

**Keyword:** Fulvic acid, Nitrification inhibitor, N leaching, Wheat productivity

## Introduction

Wheat (*Triticumae stivum* L.) is considered one of the most important cereal crops in the world and Egypt (El-Akhdar et al., 2019). Egypt's wheat production in marketing in 2018/19 was 8.45 million metric tons where the consumption was 20.1 million metric tons, so we need to increase its productivity (USDA, 2018).

Egypt lies in arid and semiarid regions which and the soils are characterized with low organic matter, high pH and high $CaCO_3$ content (Malakouti, 2008), under such conditions, soil application of the chemical nutrients are very expensive. In addition, there are several common problems related to the stressed environments. These stresses include decline of soil fertility, salt-affected soils, soil pollution, over-population growth, urban sprawl, land degradation, deterioration of natural resources, etc. (Alshaal and El-Ramady, 2017 and El-Ramady et al., 2019).

Nitrogen is one of the most important elements for plant nutrition and it is necessary for good wheat grain yield and quality. It is also one of the most mobile plant nutrients in the soil. Therefore, it is important to evaluate the use of the proper nitrogen rates as well as its leaching from the soil (Huang et al., 2018). In general, about

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50-70 % of the applied conventional chemical fertilizers get lost in in due to many environmental processes such as runoff, leaching, emissions and volatilization. Therefore, new alternatives should be used for eco-friendly, economic and organic or sustainable agriculture such as organic-, bio- and nano-fertilizers as well as slow or controlled release fertilizers (El-Ramady et al., 2018 and El-Naqma et al., 2019).

Fulvic acid is a mixture of carboxylic acids, phenolic-OH and enolic groups produced by the biodegradation of organic matter. Fulvic acids are humic acids with higher oxygen content and lower molecular weight (Bulgari et al., 2015), which can dissociate to develop negative charges (Plaza et al., 2006). Fulvic acid as an organic fertilizer is a non-toxic chelating compound that stimulates plant productivity and contributes towards cation exchange capacity of the soil (Malan, 2015 and Yang et al., 2013) demonstrated that fulvic acid is an optimum choice for the improvement of P availability and soil physicochemical conditions. Rasool et al. (2015) recommended that the application of humic acid increased wheat yield and yield components. Attia and Abd El Salam (2016) reported that high yield of wheat and preserve the environment by decreasing pollution, in addition to improvement of soil fertility and water holding capacity were obtained with the application of organic fertilizers and humic acid.

Nitrification is a microbial process that converts ammonia ($NH_3$) to nitrate ($NO_3$) via nitrite ($NO_2$) and significantly influences N availability for a plant, $NO_3$ leaching, and $N_2O$ emissions (Purkhold et al., 2000 and Strauss et al., 2014). Many new management practices and technologies can promote N use efficiency and alleviate environmental pollution, one of them, the application of fulvic acid and nitrification inhibitors (NI). The 3,4dymethylpyrazole phosphate (DMPP) was marketed in 1999 as one of the most effective NI (Zerulla et al. 2001 and Rowling et al., 2016) and has become very popular NI, particularly when added to ammonium sulfate nitrate (ASN), ammonium sulfate or urea (Trenkel, 2010). Several studies showed that the use of DMPP is a powerful tool that can be used to promote N use efficiency and reduce N losses from agricultural systems by slowing nitrification (Weiske et al., 2001; Diez-Lopez et al., 2008; Li et al., 2008 & 2009; Di and Cameron, 2011; Liu et al. 2013; Shi et al., 2016 and Yin et al., 2017). In addition, DMPP has been demonstrated to be effective in increasing crop yields and quality (Pasda et al., 2001; Fangueiro et al., 2009; Xuet al., 2014 and Abalos et al., 2014). Therefore, the aim of this investigation was to study the effect of fulvic acid and nitrification inhibitor on $NO_3$ losses, fertilizer use efficiencies and wheat productivity.

## Materials and Methods

*Description of the study site*

Two field experiments were conducted at Sakha Agriculture Research Station, Kafr El-Sheikh Governorate, Egypt, during two successive seasons (2017/18 and 2018/19) to study the effect of fulvic acid and nitrification inhibitor on $NO_3$ losses, and yield and chemical constituents of wheat.

*Treatments and experimental design*

The experimental field was prepared and then divided into 36 plots (5 m x 6 m for each). A split plot design with three replicates were used with three fulvic acid applied (*i.e* 0, 4.76 and 9.52 kg $ha^{-1}$) as main plots as well as nitrogen fertilizers as ammonium sulphate (21% N)(with or without nitrification inhibitor) at rate of 90, 135 and 180 kg N $ha^{-1}$, in sub-plots. Nitrification inhibitor 3, 4-dimethylpyrazole phosphate (DMPP) (EC 1.7 dS $m^{-1}$ and pH 4.4) was applied at 8%. The treatments could be described as follows:

| | |
|---|---|
| $T_1$ | 180 kg N $ha^{-1}$ |
| $T_2$ | 180 kg N $ha^{-1}$ with 4.76 kg fulvic acid $ha^{-1}$ |
| $T_3$ | 180 kg N $ha^{-1}$ with 9.52 kg fulvic acid $ha^{-1}$ |
| $T_4$ | 90 kg N $ha^{-1}$ with nitrification inhibitor (NI) |
| $T_5$ | 90 kg N $ha^{-1}$ with NI + 4.76 kg fulvic acid $ha^{-1}$ |
| $T_6$ | 90 kg N $ha^{-1}$ with NI + 9.52 kg fulvic acid $ha^{-1}$ |
| $T_7$ | 135 kg N $ha^{-1}$ with nitrification inhibitor (NI) |
| $T_8$ | 135 kg N $ha^{-1}$ with NI + 4.76 kg fulvic acid $ha^{-1}$ |
| $T_9$ | 135 kg N $ha^{-1}$ with NI + 9.52 kg fulvic acid $ha^{-1}$ |
| $T_{10}$ | 180 kg N $ha^{-1}$ with nitrification inhibitor (NI) |
| $T_{11}$ | 180 kg N $ha^{-1}$ with NI + 4.76 kg fulvic acid $ha^{-1}$ |
| $T_{12}$ | 180 kg N $ha^{-1}$ with NI + 9.52 kg fulvic acid $ha^{-1}$ |

*Cultural practices*

Wheat (*cv.* Masr 1) was sown at the rate of 144 kg $ha^{-1}$ on Nov. $28^{th}$, 2017 and harvested after full maturity on April, $20^{th}$, 2018 in the $1^{st}$ season. While in the $2^{nd}$ season it was sown on Nov., $19^{th}$, 2018 and harvested on April, $11^{th}$, 2019. Phosphorus applied with field preparation as a super monophosphate (18% $P_2O_5$) with a rate of 74.4 kg $ha^{-1}$; also, 120 kg $ha^{-1}$ potassium sulfate (48% $K_2O$) applied twice, with the first irrigation and before tillering stage. Other agricultural practices performed according to the Ministry of Agriculture recommendation for wheat plants in the North Delta Area.

*Soil analyses*

Soil samples were collected before planting and after harvesting from the surface layer (0-30 cm) in each plot. Samples were analyzed for their physical and chemical properties according to the standard methods outlined by Page et al. (1982) and Klute (1986). The physical and chemical characteristics of the tested soil are shown in Table 2).

*Estimating the leaching of $NO_3$ in drainage water*

One observation well was established in each experimental plot to record the leaching of the nitrate ions in the drainage water after the $2^{nd}$, $3^{rd}$ and $4^{th}$ irrigationsat the end of growing seasons. Nitrate using Kjeldahl method according to Cottenie et al. (1982).

*Data collected*

At the maturity stage, all wheat plants in one-meter square from each treatment were taken for plant characteristics. Plant biomass was measured as grain yield (Mg $ha^{-1}$), straw yield (Mg $ha^{-1}$), plant height (cm) and 1000-grain weight (g). Straw and grain samples of each treatment were oven dried at 70 °C then digested with ($H_2SO_4$+ $H_2O_2$) as described by Wolf (1982). Total N, P and K content was determined according to the methods described by Page et al. (1982), while K content was measured using flame photometer (Cottenie et al. 1982). Nutrients N, P and K content of grains and straw were determined by multiplying nutrient concentrations by dry yield biomass per plant and expressed as Kg $ha^{-1}$.

**TABLE 1. Some chemical composition of fulvic acid**

| EC (dS $m^{-1}$) | pH | OM (g $kg^{-1}$) | Total macronutrients (%) | | | Total micronutrients (ug$g^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | N | P | K | Fe | Mn | Zn | Cu |
| 1.94 | 6.88 | 10.8 | 2.15 | 2.5 | 4.00 | 418 | 260 | 219 | 21.96 |

**TABLE 2. Some physical and chemical analysis of soil in the two experimental fields**

| Soil characteristics | First season | Second season |
|---|---|---|
| Particle size distribution | **2017/18** | **2018/19** |
| Clay (%) | 50.09 | 50.12 |
| Silt (%) | 31.41 | 31.42 |
| Sand (%) | 18.50 | 18.46 |
| Texture class | **clayey** | **clayey** |
| Organic matter (g $kg^{-1}$) | 11.6 | 12.5 |
| $CaCO_3$ (g $kg^{-1}$) | 24.6 | 24.2 |
| ECe (dS $m^{-1}$) | 2.90 | 2.77 |
| pH [in soil suspension, 1:2.5] | 8.30 | 8.29 |
| Available N (mg $kg^{-1}$) | 22.56 | 22.69 |
| Available P (mg $kg^{-1}$) | 6.33 | 6.90 |
| Available K (mg $kg^{-1}$) | 270.69 | 275.89 |

The following parameters were calculated

Partial factor productivity (PP) by the equation described by Dobermann (2005)

$$PP= Y / N_f, g\ g^{-1}$$

Nutrient (nitrogen or phosphorus) accumulation efficiency (NAE) and Yield nutrient (nitrogen or phosphorus) concentration (CN) according to Weih et al. (2011)

$$NAE = N_y / N_p, g\ g^{-1}$$

$$CN = N_y / Y, g\ g^{-1}$$

Where:

Y : harvested yield (kg $ha^{-1}$).

$N_f$ : N amount fertilized (kg $ha^{-1}$).

$N_p$ : N amount in perennial plant parts (e.g. seed in cereals) (kg $ha^{-1}$).

$N_y$ : N amount in the harvested yield (kg $ha^{-1}$).

*Statistical analysis*

All data of the two growing seasons were subjected to the statistical analysis according to the methods described by Snedecor and Cochran (1980) using the CoStat package program. The differences among the means of different treatments were tested using the Least Significant Differences (LSD) at the probability of 5%.

## Results and Discussion

*Soil characteristics*

Results indicated that the application of fulvic acid, nitrogen fertilizers (with or without nitrification inhibitor) individually or combined showed a pronounced enhancement of some soil characteristics.

*Soil salinity, OM and available NPK after wheat harvesting*

The addition of fulvic acid and nitrogen fertilizers (with or without nitrification inhibitor) individually or combined increased EC, OM, available N, P and Kafter wheat harvesting in both seasons (Fig. 1-5).

Data in Fig. 1-5 showed the addition of fulvic acid increased EC, OM, available N, P and K compared with the untreated soil (without fulvic acid addition). Meanwhile, application of 9.52 kg fulvic acid $ha^{-1}$ increased mean values of EC, OM, available N, P and K from (3.11 dS $m^{-1}$, 1.30 %, 37.33 mg $kg^{-1}$, 7.50 mg $kg^{-1}$ and 299.53 mg $kg^{-1}$, respectively) with the untreated soil (without fulvic acid addition) to (3.22 dS $m^{-1}$, 1.37 %, 39.48 mg $kg^{-1}$, 7.88 mg $kg^{-1}$ and 315.05 mg $kg^{-1}$, respectively) when soil received 9.52 kg fulvic acid $ha^{-1}$. The increment of EC, OM, available N, P and K in the treatment which resaved organic acid compared to urea alone may be due to the composition of organic acid and the combination of urea with humic acid which significantly reduced urea hydrolysis (Nasima et al., 2010). These results are confirmed with those obtained by Abd El-Kader (2016) who reported that the application of mineral N with fulvic acid increased the soil OM, ECe and available NPK over that with mineral N alone.

Addition of nitrification inhibitor (DMPP 8%) with nitrogen fertilizers increased EC, OM, available N, P and K from (2.84 dS $m^{-1}$, 1.32 %, 23.93 mg $kg^{-1}$, 7.17 mg $kg^{-1}$ and 287.50 mg $kg^{-1}$, respectively) with the nitrogen fertilizers alone (without DMPP addition) to (3.49 dS $m^{-1}$, 1.37%, 55.43 mg $kg^{-1}$, 8.33 mg $kg^{-1}$ and 332.6 mg $kg^{-1}$, respectively). These results agree with Weiske et al. (2001); Diez-Lopez et al. (2008); Li et al., (2008) & (2009); Di and Cameron (2011); Liu et al. (2013); Shi et al. (2016); Yin et al. (2017) they indicated that the application of nitrification inhibitor with N fertilizer reduced N losses from soil due to slowing nitrification process.

The highest mean value of EC, OM and available N, P and K (3.53 dS $m^{-1}$, 1.42 %, 58.7 mg $kg^{-1}$, 8.8 mg $kg^{-1}$ and 352.3 mg $kg^{-1}$, respectively) were recorded from the plots received 180 kg N $ha^{-1}$ with DMPP + 9.52 kg fulvic acid $ha^{-1}$. However, the 180 kg N $ha^{-1}$ alone recorded the lowest mean values of the previous parameters (2.67 dS $m^{-1}$, 1.24%, 22.8 mg $kg^{-1}$, 6.9 mg $kg^{-1}$ and 274.1 mg $kg^{-1}$, respectively).

*Nitrate leaching in drainage water (mg $kg^{-1}$)*

Data illustrated in Table 3 revealed that the effect of fulvic acid application and nitrogen fertilizers (with and without nitrification inhibitor) individually or combined on $NO_3$-N in soil drainage water. Fulvic acid application significant decreased $NO_3$-N leaching in drainage water; results obviously showed that the drainage water from the untreated soil (without fulvic acid addition) have high rates of $NO_3$-N in both growing seasons. The decreasing of $NO_3$-N leaching in drainage water in the treatment which resaved organic acid may be due to N in the soil solution along soil profile may mainly be in $NH_4$-N form rather than $NO_3$-N form due to application of fulvic acids.

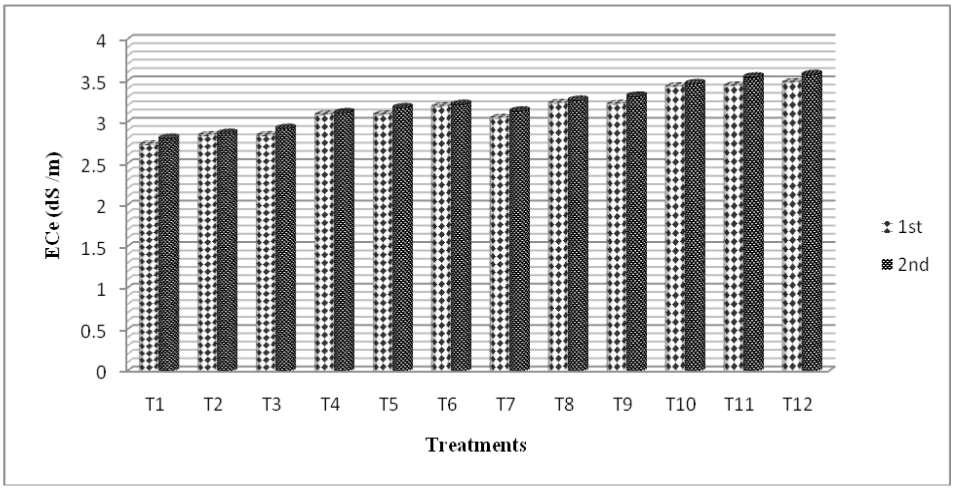



**Fig. 1. The combined effect of fulvic acid and nitrification inhibitor onECe in first and second seasons**

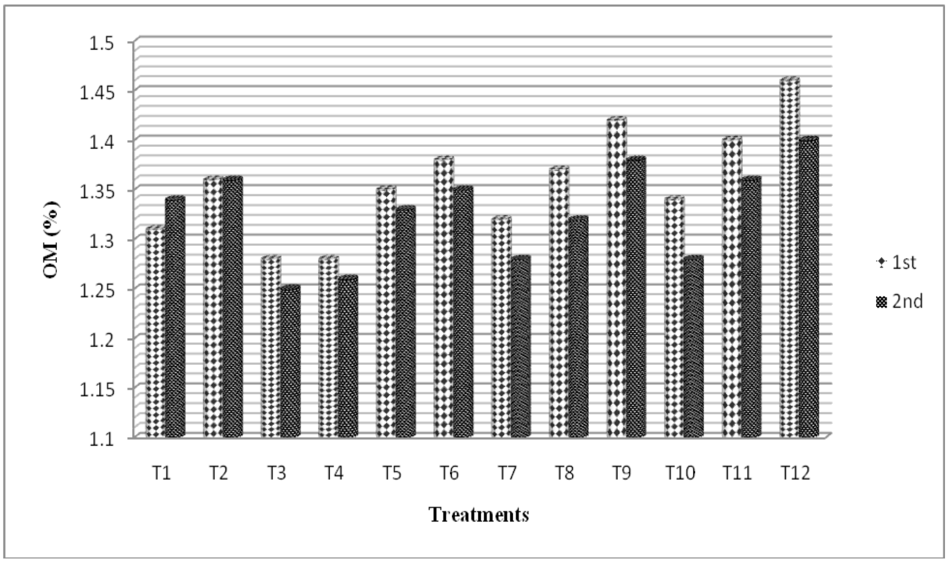



**Fig. 2. The combined effect of fulvic acid and nitrification inhibitor on OM in first and second seasons**

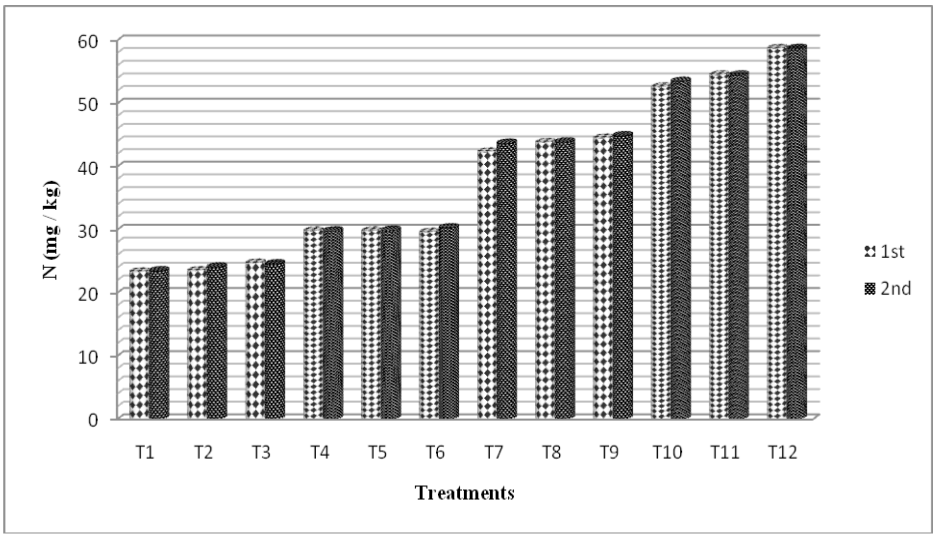



**Fig. 3. The combined effect of fulvic acid and nitrification inhibitor on soil available N in first and second seasons**

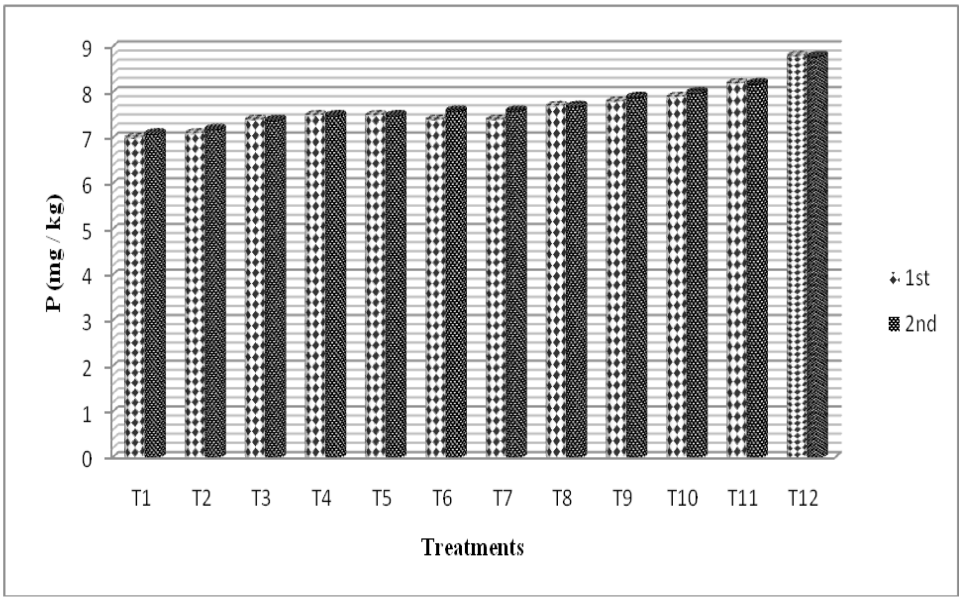



**Fig. 4. The combined effect of fulvic acid and nitrification inhibitor on soil available P in first and second seasons**

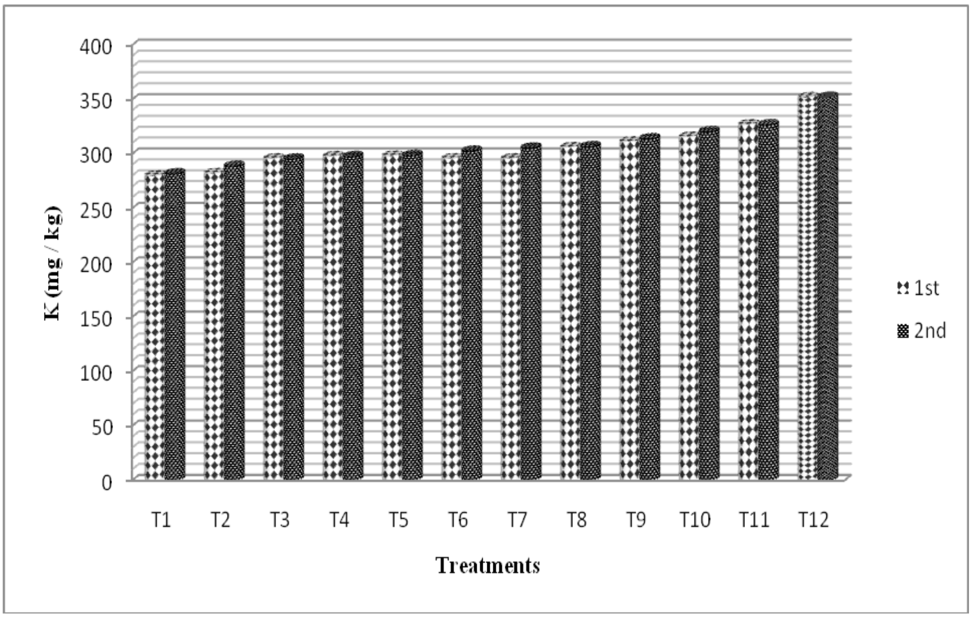



**Fig. 5. The combined effect of fulvic acid and nitrification inhibitor on soil available K in first and second seasons**

Regarding to the effect of nitrogen fertilizers combined with nitrification inhibitor (DMPP) decreased $NO_3$-N in drainage water after the 2nd, 3rd and 4th irrigations according the following descending order: 90 kg N ha$^{-1}$ combined with 8% DMPP+135 kg N ha$^{-1}$ combined with 8% DMPP+180 kg N ha$^{-1}$ combined with 8% DMPP+180 kg N ha$^{-1}$ alone. These results agreed with those obtained by Zaman et al. (2009); Chen et al. (2010) and Di & Cameron (2012). They suggested the application of nitrification inhibitors with N fertilizers as a practical way to reduce $NO_3$ leaching and $N_2O$ emissions. Concerning the effect of the interaction of different factors under investigation on $NO_3$-N concentration in drainage water, the data in most cases revealed that the highest mean values of $NO_3$-N leaching in the 1st and 2nd seasons (33.88 and 34.32 ppm, respectively) were obtained with 180 kg N ha$^{-1}$ alone after 2nd irrigation. Meanwhile, the lowest leaching values in both seasons (9.00 and7.64 ppm, respectively) were achieved in plots received 90 kg N ha$^{-1}$ combined with 8% DMPP + 9.52 kg fulvic acid ha$^{-1}$ after 4th irrigation.

**TABLE 3. The combined effect of fulvic acid and nitrification inhibitor on $NO_3$-Nleaching**

| No. of irrigation | Seasons | 1st | | | | 2nd | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $N_{fer.}$ & NI (kg N $ha^{-1}$) | Fulvic acid (kg $ha^{-1}$) | | | Mean (NI) | Fulvic acid (kg $ha^{-1}$) | | | Mean (NI) |
| | | 0 | 4.76 | 9.52 | | 0 | 4.76 | 9.52 | |
| Second irrigation (2nd) | $N_{180}$ | 33.88 | 31.4 | 29.92 | 31.73 | 34.32 | 33.84 | 33.28 | 33.81 |
| | $N_{90}$ DMPP | 15.80 | 14.96 | 14.12 | 14.96 | 19.20 | 17.92 | 13.08 | 16.73 |
| | $N_{135}$ DMPP | 22.68 | 21.28 | 20.72 | 21.56 | 21.72 | 20.84 | 20.04 | 20.87 |
| | $N_{180}$ DMPP | 25.00 | 24.36 | 23.28 | 24.21 | 23.36 | 23.72 | 22.48 | 23.19 |
| | Mean (F) | 24.34 | 23.00 | 22.01 | | 24.65 | 24.08 | 22.22 | |
| $LSD_{0.05}$ (F) | | | 1.19 | | | | 1.08 | | |
| $LSD_{0.05}$ (NI) | | | 1.75 | | | | 1.34 | | |
| Interaction (F*NI) | | | ** | | | | ** | | |
| Third irrigation (3th) | $N_{180}$ | 33.08 | 30.48 | 29.32 | 30.96 | 33.56 | 32.92 | 32.76 | 33.08 |
| | $N_{90}$ DMPP | 12.04 | 11.40 | 10.32 | 11.25 | 14.68 | 14.20 | 9.32 | 12.73 |
| | $N_{135}$ DMPP | 21.76 | 21.16 | 20.56 | 21.16 | 20.88 | 20.08 | 19.56 | 20.17 |
| | $N_{180}$ DMPP | 24.68 | 23.44 | 22.16 | 23.43 | 23.00 | 22.08 | 21.64 | 22.24 |
| | Mean (F) | 22.89 | 21.62 | 20.59 | | 23.03 | 22.32 | 20.82 | |
| $LSD_{0.05}$ (F) | | | 1.90 | | | | 1.65 | | |
| $LSD_{0.05}$ (NI) | | | 1.65 | | | | 1.12 | | |
| Interaction (F*NI) | | | ** | | | | ** | | |
| Fourth irrigation (4th) | $N_{180}$ | 32.68 | 29.96 | 28.04 | 30.23 | 33.08 | 32.72 | 31.4 | 32.40 |
| | $N_{90}$ DMPP | 10.52 | 9.76 | 9.00 | 9.76 | 13.52 | 8.40 | 7.64 | 9.85 |
| | $N_{135}$ DMPP | 21.04 | 19.8 | 19.32 | 20.05 | 19.76 | 19.04 | 18.28 | 19.03 |
| | $N_{180}$ DMPP | 23.52 | 21.56 | 20.08 | 21.72 | 20.88 | 20.96 | 19.40 | 20.41 |
| | Mean (F) | 21.94 | 20.27 | 19.11 | | 21.81 | 20.28 | 19.18 | |
| $LSD_{0.05}$ (F) | | | 1.02 | | | | 0.95 | | |
| $LSD_{0.05}$ (NI) | | | 1.22 | | | | 1.10 | | |
| Interaction (F*NI) | | | ** | | | | ** | | |

*Yield and biomass of wheat plants*

Table 4 exhibited the effect of fulvic acid application and nitrogen fertilizers (with or without nitrification inhibitor) individually or combined on wheat plants biomass. The magnitude of the enhancement of plant biomass was observed with fulvic acid application. The application of 9.52 kg fulvic acid $ha^{-1}$ increased plant biomass via. plant height (101.44 and 104.49 cm), 1000-grain weight (49.43 and 51.09 g), grain yield (6.07 and 6.34 Mg $ha^{-1}$) and straw yield (11.31 and 11.35 Mg $ha^{-1}$) in the first and second seasons, respectively following by 4.76 kg fulvic acid $ha^{-1}$ as compared to the untreated soil (without fulvic acid addition). Similar findings have been reported by Sootahar et al. (2019) who found that fulvic acids have direct and indirect involvement on plant growth due to improving soil aggregation, aeration, microbial growth, organic matter mineralization, water holding capacity and availability of macro and micronutrients. Abd El-Kader (2016) indicated that application of mineral N with fulvic acid increased the grain, straw and 100-grain weight and wheat yield compared to urea alone.

Concerning the effect of nitrogen fertilizers (with or without nitrification inhibitor) individually or combined, the nitrogen fertilizers combined with nitrification inhibitor application displayed an overall improvement in plant biomass than nitrogen fertilizers alone. The nitrogen fertilizers combined with nitrification inhibitor significantly ($p<0.05$) increased plant height (23.51 and 14.85%); 1000-grain weight (15.38 and 24.78%); grain yield (45.41 and 44.29%) and straw yield (35.79 and 46.86%) were obtained with application of 180 kg N $ha^{-1}$ combined with 8% DMPP in first and second seasons, respectively as compared to 180 kg N $ha^{-1}$ alone. These results are supported by the findings of Pasda et al. (2001); Fangueiro et al. (2009); Xu et al. (2014) and Abalos et al. (2014).

The highest mean values of plant height (110.34 and 111.42 cm); 1000-grain weight (55.60 and 61.00 g); grain yield (7.62 and 7.95 Mg $ha^{-1}$) and straw yield (13.64 and 14.02 Mg $ha^{-1}$) were recorded by 180 kg N $ha^{-1}$ combined with

8% DMPP + 9.52 kg fulvic acid $ha^{-1}$ in the first and second seasons, respectively. Nevertheless, the lowest mean values of plant height (81.25 and 87.85 cm); 1000-grain weight (44.60 and 45.50 g); grain yield (4.64 and 4.88Mg $ha^{-1}$) and straw yield (8.64 and 8.43 Mg $ha^{-1}$) were obtained with180 kg N $ha^{-1}$ alone in first and second seasons, respectively.The increment of wheat yieldand itsparameter might be due to that the fulvic acid is rich in different nutrients, while N fertilizer with DMPP led to slowly dissolve in N, in sequence conserved N from leaching and improved plant growth.

Also, data indicate that, the different rate of N-mineral fertilizers combined with nitrification inhibitor (DMPP 8%) gave better results in increasing the yield of wheat relative to N-mineral fertilizers alone, these reasons lead to decrease N-mineral fertilizers addition to soil; improve soil fertility and protect the environment from severe pollution.

*The concentration of N in grain and straw of wheat plants*

A glance on Fig. 6 and 7 illustrated that concentration of N in wheat grains and straw were affected significantly ($P<0.05$) by the application of fulvic acid and nitrogen fertilizers (with or without nitrification inhibitor) individually or combined.

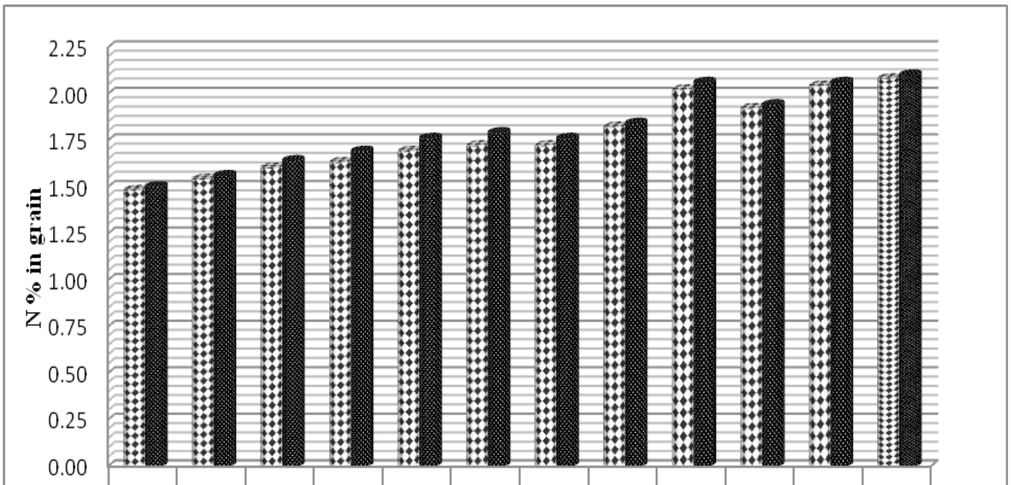
The results indicated that the concentration of N in wheat grains and straw took the same manner of yield and its components. Mean while, data reveal fulvic acid application at rate of 4.76 or/and 9.52 kg fulvic acid $ha^{-1}$ clearly increased

**TABLE 4. Effect of fulvic acids and nitrification inhibitor on some wheat parameters**

| Parameters | Seasons | 1st | | | | 2nd | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $N_{fer.}$ & NI (kg N $ha^{-1}$) | Fulvic acid (kg $ha^{-1}$) 0 | 4.76 | 9.52 | Mean (NI) | Fulvic acid (kg $ha^{-1}$) 0 | 4.76 | 9.52 | Mean (NI) |
| Plant height (cm) | $N_{180}$ | 81.25 | 86.79 | 88.90 | **85.65** | 87.85 | 92.73 | 97.68 | **92.75** |
| | $N_{90}$ DMPP | 86.00 | 88.11 | 99.00 | **91.04** | 92.05 | 93.75 | 99.40 | **95.07** |
| | $N_{135}$ DMPP | 93.75 | 103.75 | 107.52 | **101.67** | 95.50 | 107.00 | 109.44 | **103.98** |
| | $N_{180}$ DMPP | 99.00 | 108.00 | 110.34 | **105.78** | 99.60 | 108.58 | 111.42 | **106.53** |
| | **Mean (F)** | **90.00** | **96.66** | **101.44** | | **93.75** | **100.51** | **104.49** | |
| $LSD_{0.05}$ (F) | | | 0.206 | | | | 0.321 | | |
| $LSD_{0.05}$ (NI) | | | 0.083 | | | | 0.060 | | |
| Interaction (F*NI) | | | ** | | | | ** | | |
| 1000 grain weight (g) | $N_{180}$ | 44.60 | 45.10 | 45.50 | **45.07** | 45.50 | 45.90 | 46.20 | **45.87** |
| | $N_{90}$ DMPP | 45.20 | 46.20 | 47.10 | **46.17** | 46.50 | 47.20 | 47.80 | **47.17** |
| | $N_{135}$ DMPP | 47.50 | 48.90 | 49.50 | **48.63** | 47.20 | 48.65 | 49.35 | **48.40** |
| | $N_{180}$ DMPP | 49.30 | 51.10 | 55.60 | **52.00** | 54.80 | 55.90 | 61.00 | **57.23** |
| | **Mean (F)** | **46.65** | **47.83** | **49.43** | | **48.50** | **49.41** | **51.09** | |
| $LSD_{0.05}$ (F) | | | 0.401 | | | | 0.828 | | |
| $LSD_{0.05}$ (NI) | | | 0.530 | | | | 0.912 | | |
| Interaction (F*NI) | | | ** | | | | ** | | |
| Grain yield (Mg $ha^{-1}$) | $N_{180}$ | 4.64 | 5.12 | 5.28 | **5.01** | 4.88 | 5.28 | 5.47 | **5.21** |
| | $N_{90}$ DMPP | 4.78 | 5.19 | 5.28 | **5.09** | 5.05 | 5.31 | 5.62 | **5.32** |
| | $N_{135}$ DMPP | 5.09 | 5.64 | 6.09 | **5.61** | 5.40 | 5.93 | 6.33 | **5.89** |
| | $N_{180}$ DMPP | 7.09 | 7.16 | 7.62 | **7.29** | 7.19 | 7.43 | 7.95 | **7.52** |
| | **Mean (F)** | **5.40** | **5.78** | **6.07** | | **5.63** | **5.99** | **6.34** | |
| $LSD_{0.05}$ (F) | | | 0.002 | | | | 0.004 | | |
| $LSD_{0.05}$ (NI) | | | 0.004 | | | | 0.003 | | |
| Interaction (F*NI) | | | ** | | | | ** | | |
| Straw yield (Mg $ha^{-1}$) | $N_{180}$ | 8.64 | 9.07 | 9.42 | **9.04** | 8.43 | 8.64 | 8.73 | **8.60** |
| | $N_{90}$ DMPP | 10.73 | 11.07 | 11.52 | **11.11** | 11.07 | 11.28 | 11.80 | **11.38** |
| | $N_{135}$ DMPP | 11.47 | 12.26 | 10.66 | **11.46** | 11.31 | 12.57 | 10.85 | **11.57** |
| | $N_{180}$ DMPP | 10.12 | 13.09 | 13.64 | **12.28** | 10.42 | 13.45 | 14.02 | **12.63** |
| | **Mean (F)** | **10.24** | **11.37** | **11.31** | | **10.31** | **11.48** | **11.35** | |
| $LSD_{0.05}$ (F) | | | 0.004 | | | | 0.002 | | |
| $LSD_{0.05}$ (NI) | | | 0.003 | | | | 0.004 | | |
| Interaction (F*NI) | | | ** | | | | ** | | |

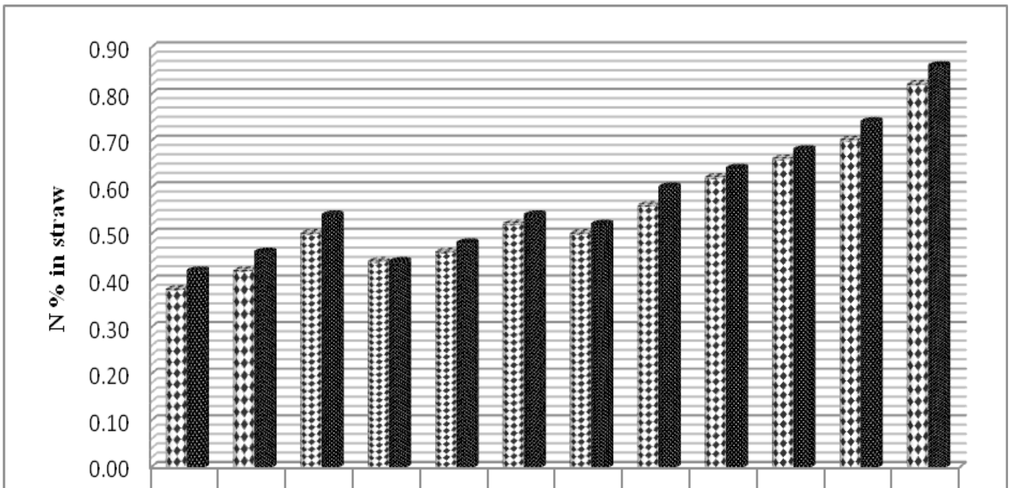
N % in wheat grains and straw. Similar findings have been reported by Sootahar et al., (2019) whofound thatfulvic acids have direct and indirect involvement on the transport of macro and micronutrients. Also, Abd El-Kader (2016) indicated that application of mineral N with fulvic acid increased the concentration of N in wheat grains and straw as compared to urea alone.

Application of N fertilizationwith nitrification inhibitor (DMPP 8%) clearly increased the N % in wheat grains and straw according the following descending order: 180 kg N $ha^{-1}$ combined with 8% DMPP+135 kg N $ha^{-1}$ combined with 8% DMPP + 90 kg N $ha^{-1}$ combined with 8% DMPP+180 kg N $ha^{-1}$ alone. This trend may be attributed to that the N fertilizer combined with DMPP led to slowly dissolve in N, in sequence conserved N from leaching and resulted in higher uptake of plant nutrients. The obtained data are confirmed with the results found by Liu et al. (2013) who revealed thatthe application of nitrification inhibitors led to increasing soil inorganic nitrogen content and shift N- $NO_3$ to N-$NH_4$, consequently increase N uptake and N use efficiency and increase wheat and maize yield.




| | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 | T10 | T11 | T12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1st | 1.48 | 1.54 | 1.60 | 1.63 | 1.69 | 1.72 | 1.72 | 1.82 | 2.02 | 1.92 | 2.04 | 2.08 |
| 2nd | 1.50 | 1.56 | 1.64 | 1.69 | 1.76 | 1.79 | 1.76 | 1.84 | 2.06 | 1.94 | 2.06 | 2.10 |

**Fig. 6. The combined effect of fulvic acid and nitrification inhibitor on N % in wheat grain**




| | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 | T10 | T11 | T12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1st | 0.38 | 0.42 | 0.50 | 0.44 | 0.46 | 0.52 | 0.50 | 0.56 | 0.62 | 0.66 | 0.70 | 0.82 |
| 2nd | 0.42 | 0.46 | 0.54 | 0.44 | 0.48 | 0.54 | 0.52 | 0.60 | 0.64 | 0.68 | 0.74 | 0.86 |

**Fig. 7. The combined effect of fulvic acid and nitrification inhibitor on N % in wheat straw**

The highest mean values of N concentration in grain and straw were (2.09 and 0.84 %, respectively) were obtained from plots received 180 kg N $ha^{-1}$ combined with 8% DMPP + 9.52 kg fulvic acid $ha^{-1}$. However, the lowest mean values of N concentration in grain and straw were (1.49 and 0.40 %, respectively) were obtained from the 180 kg N $ha^{-1}$ alone.

*Nitrogen uptake by wheat (kg $fed^{-1}$)*

The nitrogen uptake by the grain and straw of wheat were significantly affected by the application of fulvic acid, nitrogen fertilizers (with or without nitrification inhibitor) as well as their interaction (Table 5). These results are extracted from the grain and straw yield (Table 4) and N concentration in grain and straw (Fig. 6 and 7). Fulvic acid application significant increased N content of grain, straw and total N uptake by wheat. The application of 9.52 kg fulvic acid $ha^{-1}$ increased N content of grain (109.47 and 106.65 kg N $ha^{-1}$), N content of straw (67.21 and 64.76 kg N $ha^{-1}$) and total N uptake by wheat (176.67 and 171.41 kg N $ha^{-1}$) in first and second seasons, respectively as compared to the untreated soil (without fulvic acid addition). The increment of N content and uptake may result in the application of fulvic acids (Sootahar et al., 2019) or humic acid (Asal et al., 2015) due to enhance of root growth and macro and micronutrients uptake. These results agree with those obtained by Abd El-Kader (2016) indicated that urea coating with fulvic acid increased the N contents in grain and straw and total N uptake of wheat yield compared to urea alone.

In addition, application of N fertilization combined with nitrification inhibitor (DMPP 8%) clearly increased the N content of grain, straw and total N uptake by wheat according the following descending order: 180 kg N $ha^{-1}$ combined with 8% DMPP > 135 kg N $ha^{-1}$ combined with 8% DMPP > 90 kg N $ha^{-1}$ combined with 8% DMPP > 180 kg N $ha^{-1}$ alone. This trend may be attributed to that the N fertilizer combined with DMPP led to slowly dissolve in N, in sequence conserved N from leaching and resulted in higher uptake of plant nutrients.These results agreed with those obtained by Liu et al. (2013) found that the application of nitrification inhibitors led to increasing soil inorganic nitrogen content and shift N- $NO_3$ to N-$NH_4$, consequently increase N uptake and N use efficiency and increase wheat and maize yield.

**TABLE 5. The combined effect of fulvic acid and nitrification inhibitor on N content of wheat grain and straw**

| Parameters | Seasons | 1st | | | | 2nd | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $N_{fer.}$ & NI (kg N $ha^{-1}$) | Fulvic acid (Mg $ha^{-1}$) 0 | 4.76 | 9.52 | Mean (NI) | Fulvic acid (Mg $ha^{-1}$) 0 | 4.76 | 9.52 | Mean (NI) |
| N content of grain (Mg $ha^{-1}$) | $N_{180}$ | 68.69 | 73.19 | 82.43 | **74.77** | 73.19 | 70.94 | 80.62 | **74.92** |
| | $N_{90}$ DMPP | 77.98 | 85.28 | 93.42 | **85.56** | 85.28 | 81.63 | 90.55 | **85.82** |
| | $N_{135}$ DMPP | 87.61 | 95.09 | 109.05 | **97.25** | 95.09 | 91.35 | 105.86 | **97.43** |
| | $N_{180}$ DMPP | 136.18 | 139.45 | 152.97 | **142.87** | 139.45 | 137.81 | 149.56 | **142.27** |
| | **Mean (F)** | **92.62** | **98.25** | **109.47** | | **98.25** | **95.43** | **106.65** | |
| $LSD_{0.05}$ (F) | | | 0.733 | | | | 0.728 | | |
| $LSD_{0.05}$ (NI) | | | 0.101 | | | | 0.104 | | |
| Interaction (F*NI) | | | ** | | | | ** | | |
| N content of straw (Mg $ha^{-1}$) | $N_{180}$ | 32.84 | 35.39 | 39.75 | **35.99** | 35.39 | 34.11 | 38.92 | **36.14** |
| | $N_{90}$ DMPP | 47.24 | 48.70 | 54.16 | **50.03** | 48.70 | 47.97 | 52.54 | **49.73** |
| | $N_{135}$ DMPP | 57.36 | 58.79 | 75.40 | **63.85** | 58.79 | 58.08 | 72.03 | **62.97** |
| | $N_{180}$ DMPP | 66.77 | 70.89 | 99.51 | **79.06** | 70.89 | 68.83 | 95.58 | **78.43** |
| | **Mean (F)** | **51.05** | **53.44** | **67.21** | | **53.44** | **52.25** | **64.76** | |
| $LSD_{0.05}$ (F) | | | 0.716 | | | | 0.845 | | |
| $LSD_{0.05}$ (NI) | | | 0.254 | | | | 0.282 | | |
| Interaction (F*NI) | | | ** | | | | ** | | |
| Total N uptake (Mg $ha^{-1}$) | $N_{180}$ | 101.53 | 108.58 | 122.18 | **110.76** | 108.58 | 105.06 | 119.54 | **111.06** |
| | $N_{90}$ DMPP | 125.22 | 133.98 | 147.57 | **135.59** | 133.98 | 129.60 | 143.09 | **135.55** |
| | $N_{135}$ DMPP | 144.97 | 153.88 | 184.45 | **161.10** | 153.88 | 149.43 | 177.88 | **160.40** |
| | $N_{180}$ DMPP | 202.95 | 210.34 | 252.49 | **221.92** | 210.34 | 206.64 | 245.14 | **220.71** |
| | **Mean (F)** | **143.67** | **151.70** | **176.67** | | **151.70** | **147.68** | **171.41** | |
| $LSD_{0.05}$ (F) | | | 1.394 | | | | 1.521 | | |

The highest mean values of N content of grain (152.97 and 149.56 kg N ha$^{-1}$); N content of straw (99.51 and 95.58 kg N ha$^{-1}$) and total N uptake by wheat (252.49 and 245.14 kg N ha$^{-1}$) were recorded by 180 kg N ha$^{-1}$ combined with 8% DMPP + 9.52 kg fulvic acid ha$^{-1}$ in first and second seasons, respectively. While the lowest mean values were observed with 180 kg N ha$^{-1}$ alone.

*Partial factor productivity (PP), Nitrogen accumulation efficiency (NAE) and Yield nitrogen concentration (CN)*

Data presented in Table 6 showed the effect of the application of fulvic acid, nitrogen fertilizers (with or without nitrification inhibitor) and their interaction on Partial factor productivity (PP), Nitrogen accumulation efficiency (NAE) and Yield nitrogen concentration (CN). Application of fulvic acid at 9.52 kg ha$^{-1}$ significantly affected PP, NAE and CN in both seasons with value of 43.88, 16.15, 0.0105g g$^{-1}$ and 45.97, 16.03, 0.0109 g g$^{-1}$ in the first and second season, respectively. Hence, the untreated soil (without fulvic acid addition) recorded the lowest mean values in both seasons. N fertilization combined with nitrification inhibitor (DMPP 8%) clearly increased the PP according the following descending order: 90 kg N ha$^{-1}$ combined with 8% DMPP > 135 kg N ha$^{-1}$ combined with 8% DMPP > 180 kg N ha$^{-1}$ combined with 8% DMPP > 180 kg N ha$^{-1}$ alone. While NAE and CN according the following descending order: 180 kg N ha$^{-1}$ combined with 8% DMPP > 135 kg N ha$^{-1}$ combined with 8% DMPP > 90 kg N ha$^{-1}$ combined with 8% DMPP > 180 kg N ha$^{-1}$ alone.

Concerning the interaction, effect data revealed that high significant interactions between fulvic acid and nitrogen fertilizers (with or without nitrification inhibitor) on PP, NAE and CN were obtained. The highest mean values of PP 58.71 and 62.41 g g$^{-1}$ in the first and second season, respectively were recorded under 90 kg N ha$^{-1}$ combined with 8% DMPP + 9.52 kg fulvic acid ha$^{-1}$. While the highest mean values of NAE and CN 17.06, 0.0127g g$^{-1}$ and 17.22, 0.0131g g$^{-1}$ in the first and second season, respectively were recorded under 180 kg N ha$^{-1}$ combined with 8% DMPP + 9.52 kg fulvic acid ha$^{-1}$. The lowest mean values were achieved under 180 kg N ha$^{-1}$ alone.

| | | |
|---|---|---|
| $LSD_{0.05}$ (NI) | 0.336 | 0.368 |
| Interaction (F*NI) | ** | ** |

**TABLE 6. The combined effect of fulvic acid and nitrification inhibitor on Partial factor productivity (PP), Nitrogen accumulation efficiency (NAE) and Yield nitrogen concentration (CN)**

| Parameters | Seasons | 1st | | | | 2nd | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $N_{fer.}$ & NI (kg N ha$^{-1}$) | Fulvic acid (Mg ha$^{-1}$) 0 | 4.76 | 9.52 | Mean (NI) | Fulvic acid (Mg ha$^{-1}$) 0 | 4.76 | 9.52 | Mean (NI) |
| PP (g g$^{-1}$) | $N_{180}$ | 25.78 | 28.43 | 29.35 | **27.85** | 27.11 | 29.35 | 30.41 | **28.96** |
| | $N_{90}$ DMPP | 53.15 | 57.65 | 58.71 | **56.50** | 56.06 | 58.97 | 62.41 | **59.15** |
| | $N_{135}$ DMPP | 37.73 | 41.78 | 45.13 | **41.55** | 40.02 | 43.90 | 46.89 | **43.60** |
| | $N_{180}$ DMPP | 39.40 | 39.80 | 42.31 | **40.50** | 39.93 | 41.25 | 44.16 | **41.78** |
| | **Mean (F)** | **39.02** | **41.91** | **43.88** | | **40.78** | **43.37** | **45.97** | |
| $LSD_{0.05}$ (F) | | 0.181 | | | | 0.178 | | | |
| $LSD_{0.05}$ (NI) | | 0.099 | | | | 0.01 | | | |
| Interaction (F*NI) | | ** | | | | ** | | | |
| NAE (g g$^{-1}$) | $N_{180}$ | 14.78 | 14.83 | 15.57 | **15.06** | 14.83 | 14.82 | 15.25 | **14.97** |
| | $N_{90}$ DMPP | 16.05 | 15.80 | 16.59 | **16.15** | 15.71 | 15.80 | 16.34 | **15.95** |
| | $N_{135}$ DMPP | 16.55 | 16.68 | 15.37 | **16.20** | 16.18 | 16.91 | 15.33 | **16.14** |
| | $N_{180}$ DMPP | 15.90 | 16.27 | 17.06 | **16.41** | 15.08 | 16.50 | 17.22 | **16.27** |
| | **Mean (F)** | **15.57** | **15.90** | **16.15** | | **15.45** | **16.01** | **16.03** | |
| $LSD_{0.05}$ (F) | | 0.075 | | | | 0.064 | | | |
| $LSD_{0.05}$ (NI) | | 0.012 | | | | 0.014 | | | |
| Interaction (F*NI) | | ** | | | | ** | | | |
| CN (g g$^{-1}$) | $N_{180}$ | 0.0076 | 0.0082 | 0.0090 | **0.0083** | 0.0082 | 0.0088 | 0.0096 | **0.0089** |
| | $N_{90}$ DMPP | 0.0081 | 0.0085 | 0.0090 | **0.0085** | 0.0083 | 0.0089 | 0.0094 | **0.0089** |
| | $N_{135}$ DMPP | 0.0088 | 0.0096 | 0.0113 | **0.0099** | 0.0092 | 0.0100 | 0.0116 | **0.0103** |
| | $N_{180}$ DMPP | 0.0118 | 0.0117 | 0.0127 | **0.0121** | 0.0119 | 0.0121 | 0.0131 | **0.0124** |
| | **Mean (F)** | **0.0091** | **0.0095** | **0.0105** | | **0.0094** | **0.0099** | **0.0109** | |
| $LSD_{0.05}$ (F) | | 0.0004 | | | | 0.0002 | | | |

## Conclusion

We can conclude from this study that the fulvic acid application and N fertilizer with DMPP as nitrification inhibitor caused reduction of nitrate losses as well as improved wheat productivity. In addition, fulvic acid combined with N fertilizer with DMPP was more effective on wheat growth and yield as well as nutrients uptake. Therefore, a combined application of them increases the nutrient content in the soil, consequently decreases $NO_3$-Nleaching and protects the environment from severe pollution.

## References

Abalos, D.; S. Jeffery; A. Sanz-Cobena; G. Guardia and A. Vallejo (2014). Meta-analysis of the effect of urease and nitrification inhibitors on crop productivity and nitrogen use efficiency. *Agriculture Ecosystems & Environment,* **189**: 136-144.

Abd EL-Kader N. (2016). The Combined Effect of Urea and Organic Acid on Some Soil Properties, Yield and Nutrient Uptake by Wheat Crop. *Egypt. J. Soil Sci.* **56** (2), 281-294.

Asal, M. W.; E. A. Badr; O. M. Ibrahim and E. G. Ghalab (2015). Can humic acid replace part of the applied mineral fertilizers? A study on two wheat cultivars grown under calcareous soil conditions. International *Journal of ChemTech Research,* **8,** (9), 20-26.

Alshaal T. and H. El-Ramady (2017) Sustainable agriculture: towards holistic overview. *J. Sus. Agric. Sci.***43** (2), 65- 67. DOI: 10.21608/JSAS.2017.3609

Attia, M. A. and A. A. Abd El Salam (2016). Response of wheat 'Triticumaestivum L.' to humic acid and organic fertilizer applicationunder varying Siwa Oasis conditions. *J. of Agriculture and Veterinary Science* (IOSR-JAVS)Vol. 9, Issue 9 Ver. I: 81-86.

Bulgari R.; G. Cocetta; A. Trivellini; P. Vernieri and A. Ferrante (2015). Bio stimulants and crop responses: a review. *Biol. Agric. Hortic.* **31**(1),1–17.

Chen D.; H. C. Suter; A. Islam and R. Edis (2010) Influence of nitrification inhibitors on nitrification and nitrous oxide ($N_2O$) emission from a clay loam soil fertilized with urea. *Soil Biol. Biochem.* **42**, 660–664.

Cottenie, A., Verlo, M., Kjekens, L. and Camerlynch, R. (1982) *Chemical Analysis of Plant and Soil.* Laboratory of Analytical Agrochemistry. State University, Gent, Belgium, (42), 80-284.



Di H. J. and K. C. Cameron (2011). Inhibition of ammonium oxidation by a liquid formulation of 3,4-dimethylpyrazole phosphate (DMPP) compared with a dicyandiamide (DCD) solution in six new Zealand grazed grassland soils. *J. Soils Sediments,* **11**,1032–1039.

Di H. J. and K. C. Cameron (2012) How does the application of different nitrification inhibitors affect nitrous oxide emissions and nitrate leaching from cow urine in grazed pastures? *Soil Use Manage.* **28**, 54-61.

Diez-Lopez, J. A.; P. Hernaiz-Algarra; M. Arauzo-Sanchez and I. Carrasco-Martin (2008) Effect of a nitrification inhibitor (DMPP) on nitrate leaching and maize yield during two growing seasons. *Spanish Journal of Agricultural Research,* **6**, 294-303.

Dobermann A. R. (2005) Nitrogen use efficiency - state of the art. Agronomy & Horticulture Faculty Publications 316.

El-Akhdar I. A.; M. Elshikh; Nanis G. Allam and C. Staehelin (2019) The Potential Use of Microbial Inocula for Improving Wheat Productivity in Saline Soils.*Env. Biodiv. Soil Security,* Vol. **3,** 131 – 146. DOI: 10.21608/JENVBS.2019.13580.1062

El-NaqmaKholoud A.; R. A. El-Dissoky and M. A. Aiad (2019) Impact of Green Manure, Mineral and Bio Fertilizers on Soil Fertility, Onion-maize Productivity and N-use Efficiency. *Env. Biodiv. Soil Security,* Vol. **3**, 11- 28.DOI: 10.21608/JENVBS.2019.7507.1051

El-Ramady H.; M. Abowaly; F. Elbehiry; A. E. D.Omara; T. A. Elsakhawy; S. Mohamed; A. Belal; H. Elbasiouny and Z. F. Abdalla (2019) Stressful Environments and Sustainable Soil Management: A Case Study of Kafr El-Sheikh, Egypt. *Environment, Biodiversity and Soil Security,* **3,** 41-50.DOI: 10.21608/JENVBS.2019.17750.1070

Fangueiro D.; A. Fernandes, J. Coutinho, N. Moreira and H. Trindade (2009) Influence of two nitrification inhibitors (DCD and DMPP) on annual ryegrass yield and soil mineral N dynamics after incorporation with cattle slurry. *Commun Soil Sci. Plan,* **40**, 3387–3398.

Huang P.; J. Zhang; A. Zhu; X. Li; D. Ma; X. Xin; C. Zhang; S. Wu; G. Garland and E. I. P. Pereira (2018) Nitrate accumulation and Leaching potential reduced by coupled water and nitrogen management in the Huang-Huai-Hai Plain. *Sciences of the Total Environment,* **610 611**, 1020 1028.

Klute, A. Ed. (1986) *"Methods of soil analysis"*. No. 9 Part 1, Amer. Soc. Agron., Inc. Madison, Wisconsin, USA.

Li H.; Y. Chen; X. Liang; Y. Lian and W. Li (2009). Mineral-nitrogen Leaching and Ammonia Volatilization from a Rice–Rapeseed System as Affected by 3,4-Dimethylpyrazole Phosphate. *Journal of Environmental Quality,* Vol. **38** (9), 2131-2137.

Li, H.; X. Liang; Y. Chen; Y. Lian; G. Tian and W. Ni (2008). Effect of nitrification inhibitor DMPP on nitrogen leaching, nitrifying organisms, and enzyme activities in a rice-oilseed rape cropping system. *Journal of Environmental Sciences-China,* **20**, 149-155.

Liu C.; K. Wang and X. Zheng (2013). Effects of nitrification inhibitors (DCD and DMPP) on nitrous oxide emission, crop yield and nitrogen uptake in a wheat–maize cropping system. *Biogeo Sciences,* **10**, 2427–2437.

Malakouti, M.J. (2008) The Effect of micronutrients in ensuring efficient use of macronutrients.*Turk. J. For.* **32**, 215-220.

Malan, C. (2015) Review: humic and fulvic acids. A Practical Approach. In Sustainable soil management symposium. Stellenbosch, 5-6 November 2015, Agrilibrium Publisher.

Nasima, J.; M. Y. Khanif and K. A. Dharejo (2010). Maize response to biodegradable polymer and urease Inhibitor coated urea. *Intl. J. Agric. Biol.* **12**, 773-776.

Page, A.L., R. H. Miller and D.R. Keeney (1982). Methods of Soil Analysis". Part 2: Chemical and microbiological properties. second edition, Amer Soc. Of Agron. Madison, Wisconsin, USA. properties. *Agron. J.* **98**, 471-1478.

Pasda G.; R. Hähndel and W. Zerulla (2001). Effect of fertilizers with the new nitrification inhibitor DMPP (3,4-dimethylpyrazole phosphate) on yield and quality of agricultural and horticultural crops. *Biol. Fertil. Soils,* **34**, 85– 97.

Plaza, C.; G. Brunetti; N. Senesi and A. Polo (2006). Molecular and quantitative analysis of metal ion binding to humic acids from sewage sludge and sludge amended soil by fluorescence spectroscopy. *Environ. Sci. Technol.,* **40**, 917-923.

Purkhold U.; A. Pommerening-Roser; S. Juretschko; M. C. Schmid; H. P. Koops and M. Wagner (2000) Phylogeny of all recognized species of ammonia oxidizers based on comparative 16S rRNA and amo A sequence analysis implications for molecular diversity surveys. *Appl Environ Microbiol.* **66**, 5368–5382.

Rasool G.; A. J. Wahla; M. Nawaz and M. A. Rehman (2015). Determination And Evaluation Of The Effect Of Different Doses Of Humic Acid On The Growth And Yield Of Wheat (*Triticum aestivum* L.). *Journal of Agriculture and Veterinary Science,* (IOSR-JAVS); Vol **8**, Issue 2, Ver. I, PP 05-07.

Rowlings D. W.; C. Scheer; S. Liu and P. R. Grace (2016). Annual nitrogen dynamics and urea fertilizer recoveries from a dairy pasture using 15N; effect of nitrification inhibitor DMPP and reduced application rates. *Agric. Ecosyst. Environ.* **216**, 216–225.

Shi X.; Hu H-W.; C. Müller; He J-Z; D. Chen and H. C. Suter (2016). Effects of the nitrification inhibitor 3,4-dimethylpyrazole phosphate on nitrification and nitrifiers in two contrasting agricultural soils. *Appl. Environ. Microbiol.* **82**, 5236 –5248.

Cottenie, A.; M. Ver Loo; L. Mjkiekens; G. Velghe and R. Comertynck, (1982) *Chemical Analysis of Plant and Soil.* Lab. Anal. and Agrochem. State Univ., Gent., Belgium, Chapter 2 and 3, pp. 14-54.

Snedecor, G. W. and W. G. Cochran, (1980) *Statistical Methods,* seventh ed. The Iowa State Univ. Press, Ames. Iowa, USA, pp. 1–507.

Sootahar M. K.; X. Zeng ; S. Su; Y. Wang; L. Bai ; Y. Zhang; T. Li and X. Zhang (2019) The Effect of Fulvic Acids Derived from Different Materials on Changing Properties of Albic Black Soil in the Northeast Plain of China. *Molecules,* **24**, 1535: 1-12.

Strauss S. L.; C. L. Reardon and M. Mazzola (2014) The response of ammonia oxidizer activity and community structure to fertilizer amendment of orchard soils. *Soil Biol. Biochem.* **68**, 410–418.

Trenkel, M. E. (2010). Slow- and controlled- release and stabilized fertilizers: an option for enhancing nutrient efficiency in agriculture. IFA., Paris (France).

USDA, (2018). Wheat and Corn Imports Hold Steady Despite Increased Local Production. GAIN Report Number: EG18020: 1-9.

Weih M.; L. Asplund and G. Bergkvist (2011) Assessment of nutrient use in annual and perennial crops: a functional concept for analyzing nitrogen use efficiency. *Plant Soil,* **339**, 513–520.

Weiske, A.; G. Benckiser; T. Herbert and J. C. G. Ottow (2001) Influence of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) in comparison to dicyandiamide (DCD) on nitrous oxide emissions, carbon dioxide fluxes and methane oxidation during 3 years of repeated application in field experiments. *Biology and Fertility of Soils,* **34**, 109-117.

Wolf, (1982) A comprehensive system of leaf analysis and it is use for diagnosing crop nutrient status. *Communic Soil Sci. and Plant Analysis,* **13**, 1035-1059.

Xu L.; H. Chen; J. Xu; J. Yang; X. Li; M. Liu; J. Jiao; F. Hu and H. Li (2014) Nitrogen transformation and plant growth in response to different urea application methods and the addition of DMPP. *J. Plant Nutr. Soil Sci.* **177,** 271–277.

Yang, S.; Z. Zhang; L. Cong; X. Wang and S. Shi (2013) Effect of fulvic acid on the phosphorus availability in acid soil. *Journal of Soil Science and Plant Nutrition,* **13** (3), 526-533.

Yin S.; X. Zhang; Z. Jiang; P. Zhu; C. Li and C. Liu (2017) Inhibitory Effects of 3,4-Dimethylpyrazole Phosphate on $CH_4$ and $N_2O$ Emissions in Paddy Fields of Subtropical China. *Int. J. Environ. Res. Public Health,* **2017**, **14,** 1177: 1-12.

Zaman M.; S. Saggar; J. D. Blennerhassett and J. Singh (2009) Effect of urease and nitrification inhibitors on N transformation, gaseous emissions of ammonia and nitrous oxide, pasture yield and N uptake in grazed pasture system. *Soil Biol.Biochem,* **41**, 1270 -1280.

Zerulla W.; T. Barth; J. Dressel; K. Erhardt; K. Horchler von Locquenghien; G. Pasda; M. Rädle and A. H. Wissemeier (2001) 3,4-Dimethylpyrazole phosphate (DMPP) a new nitrification inhibitor for agriculture and horticulture. *Biol Fertil Soils,* **34**,79 – 84.