Indian Journal of Agricultural Sciences **87** (4): 479–84, April 2017/Article
https://doi.org/10.56093/ijas.v87i4.69359

Induction of changes by nitrification inhibitor and nitrogen source on vegetative growth, physiological processes and biochemical constituents of Kinnow mandarin

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Received: 22 December 2014; Accepted: 16 September 2016

ABSTRACT

A field experiment was conducted on two-year-old Kinnow mandarin plant to find out effect of different nitrogen sources and nitrification inhibitors on vegetative growth, physiological processes and biochemical constituents of Kinnow mandarin. There were thirteen treatments comprising four nitrogen sources, viz. ammonium sulphate (AS), calcium nitrate (CN), mixture of ammonium sulphate + calcium nitrate and urea, two nitrification inhibitor, viz. Dicyandiamide (DCD) 5% of fertilizers, meliacins (M) 0.1% of fertilizers and control. The increase in tree height was recorded significantly higher in plants treated with AS + DCD (44.05%); whereas, tree spread E-W (77.33%), tree spread N-S (66.03%), specific leaf area (123.86 cm^2/g) and shoot growth rate (247.39%) was found maximum in AS + M. In the plants applied with AS + DCD registered significantly maximum values of chlorophyll (a, b and total) content, photosynthetic rate and stomatal conductance. However, transpiration rate was found maximum under treatment AS + M when applied during winter and summer in split doses. Ammonium sulphate treated with DCD produced statistically highest total soluble sugar (9.22, 9.78 and 9.40% leaf fresh wt) and soluble proteins (74.80, 76.49 and 71.96 mg/g leaf dry wt) during winter, autumn and summer, respectively followed by ammonium sulphate treated with meliacins. The ammonium sulphate and urea as source of N along with nitrification inhibitor have a strong impact on growth and physio-biochemical parameters on Kinnow plants; thus, improved the performance of Kinnow plants under above natural pH soil conditions.

Key words: Kinnow, Nitrogen, Nitrification inhibitor, Nitrogen sources

Citrus occupies a prominent position in the fruit industry. Presently, the Kinnow mandarin is the leading citrus cultivar with 70% share in total citrus production of India. It requires several nutrients to grow, but it did not show any visible sign of deficiency. However, citrus require frequent applications of soluble nitrogen fertilizers at high annual rates to ensure sufficient vegetative growth. The nitrogen has more influence on the growth, yield and quality of citrus than any other single plant nutrient. It is an essential ingredient of chlorophyll, proteins, growth hormones and enzymes and is a building block for fruit production (Huett 1996). Nitrogen is taken up by plants as both ammonium (NH_4^+) and nitrate (NO_3^-). Physiologically, when NO_3^- is taken up by plants, there is a simultaneous uptake of protons (H^+), resulting in an increase in rhizospheric pH. Conversely, when NH_4^+ is taken up, the H^+ is released into the rhizosphere, resulting in a decrease in rhizospheric pH (Marschner 1995).

Ammonium and nitrate are the two main forms taken by the plant roots. Nitrification is generally rapid in upland soils of citrus orchards in which the major form of nitrogen is nitrate. Ammonium and nitrate differ greatly in their ionic nature. Nitrate is the most oxidized and ammonium is the most reduced form of nitrogen. Therefore, these ions produce different physiological responses in Kinnow.

Serna *et al*. (1992) found that citrus seedlings absorbed NH_4^+ at a higher rate than NO_3^- N. Losses through leaching and/or volatilization are the main causes of low efficiency of applied N-fertilizer. Khokhar *et al.* (2012) recommended adding nitrogen as ammonium sulphate, which is less susceptible to leaching and more efficient at sandy and high pH conditions in Kinnow mandarin. Fertilizers which are treated with nitrification inhibitors synchronize nutrient release patterns and therefore optimize nutrient uptake efficiency while reducing nutrient losses to the environment. Hence, inhibitors of nitrification have been used in attempts to increase the efficiency of fertilizer

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nitrogen by various crops in conditions where losses by denitrification or leaching are high (Prasad *et al*.1971). Nitrification is the biochemical oxidation of ammonium to nitrate. *Nitrosomonas* converts ammonium to nitrite by the action of ammonia mono-oxygenase enzyme, while *Nitrobacter* oxidizes nitrite to nitrate. Nitrification inhibition is the inactivation of ammonia mono oxygenase enzyme under the chemical action of some nitrification inhibitor. A number of chemicals have been used to inhibit nitrification in soil.

Dicyandiamide (DCD) is most commonly tested in laboratory and field experiments. There are several nitrification inhibitors of plant origin and are highly cost effective such as neem (*Azadirachta indica*), *karanj* (*Pongamia glabra*), pyrethrum and Norway spurce (*Picea abies* L.) *etc*. The use of a small quantity of neem oil can serve the purpose and may be used successfully for the coating of urea. But not all the chemical components (group of compounds) of neem oil have nitrification-inhibiting properties. The major components in neem oil are free fatty acid (FFA), pure oil, meliacins, saturated and unsaturated fractions. Kumar *et al*. (2007) found in a soil incubation experiment that the meliacins content in neem oil directly affected the nitrification inhibition.

In this regards, little information is available on Kinnow mandarin response to the different nitrogen sources and nitrification inhibitors. We hypothesized that the application of different nitrogen sources and nitrification inhibitors in 'Kinnow' may influence the vegetative, physiological and biochemical constituent of plant. Therefore, the present study was conducted to investigate the effect of different nitrogen sources and nitrification inhibitors on vegetative growth, physiological processes and biochemical constituents of Kinnow mandarins. The information collected will be very beneficial for scientists, extension workers and citrus growers.

MATERIALS AND METHODS

The field experiment with two-year-old Kinnow on *Jatti khatti* rootstock plants was carried out during 2011-12 at the Todapur Orchard of Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute, New Delhiis situated between the latitudes of 28° 38' 22" N and 38° 39' 05" N and longitudes of 77° 9' 45" E and 77° 10' 24"E at an average elevation of 228.61 m above the mean sea level. Climate of Delhi is categorized as semi-arid, subtropical with hot dry summer and cold winter and it falls in the Agro-eco-region-IV. The maximum and minimum temperature during the experiment was 44.2 and 1.7°C. The total rainfall received during the experiment was 689.8 mm. Soils of IARI represent a typical alluvium profile of Yamuna origin. The pH of the experimental field was between 7.6 to 7.8.

The experiment comprised four nitrogen sources (ammonium sulphate as ammonical form, calcium nitrate as nitrate form, mixture of ammonium sulphate and calcium nitrate as nitrate and ammonical form and urea), two nitrification inhibitor (Dicyandiamide @ 5% of fertilizers and meliacin @ 0.1% of fertilizers) and one control. Total thirteen treatment combinations includes T_1= control, T_2= ammonium sulphate (AS), T_3= calcium nitrate (CN), T_4= ammonium sulphate + calcium nitrate, T_5= Urea (UR), T_6= ammonium sulphate (AS) + dicyandiamide (DCD), T_7= ammonium sulphate + meliacins (M), T_8= calcium nitrate (CN) + dicyandiamide, T_9= calcium nitrate + meliacins, T_{10}= ammonium sulphate + calcium nitrate + dicyandiamide, T_{11}= ammonium sulphate + calcium nitrate + meliacins, T_{12}= Urea (UR) + dicyandiamide and T_{13}= urea + meliacins. Recommended fertilizers dose was applied in three splits, i.e. during winter season in September (75 g N : 37.5g P : 52.5g K/plant), during Autumn season in February (150g N : 75g P : 105g K/plant) and during rainy season in June (75g N : 37.5g P : 52.5g K/plant). Nitrification inhibitors mixed with different nitrogenous fertilizers before application and then applied in the field by ring method. The experiment was laid out in randomized block design and replicated thrice. Experimental unit had two plants per treatment. Kinnow orchard was installed with online drip irrigation system. The control head of the system consisted of sand filter, flow control valve, screen filter, pressure gauges etc. The lateral lines were placed along the Kinnow row having four online emitters of four litres per hour (4 l/hr) capacity surrounding the tree. Irrigation was scheduled daily as per consumptive water requirement calculated as per formula given below;

Table 1 Effect of different nitrogen sources with or without nitrification inhibitors on shoot growth rate (%) and specific leaf area (cm^2/g) in Kinnow mandarin

Treatment	Shoot growth rate (%)	Specific leaf area (cm^2/g)
T_1 (Control)	73.89 ± 7.44^{f}	69.75 ± 2.67^{f}
T_2 (AS)	143.78 ± 8.33^{de}	89.35 ± 4.52^{cde}
T_3 (CN)	123.60 ± 12.93^{e}	82.01 ± 3.63^{e}
T_4 (AS+ CN)	125.18 ± 6.64^{e}	85.85 ± 3.00^{cde}
T_5 (UR)	166.44 ± 9.39^{d}	89.46 ± 3.87^{cde}
T_6 (AS+DCD)	238.54 ± 9.07^{a}	119.74 ± 2.95^{ab}
T_7 (AS+M)	247.39 ± 3.71^{a}	123.86 ± 3.69^{a}
T_8 (CN+DCD)	128.28 ± 4.43^{e}	83.95 ± 2.51^{de}
T_9 (CN+M)	119.72 ± 5.63^{e}	83.65 ± 2.43^{de}
T_{10} (AS+CN+DCD)	195.89 ± 12.91^{c}	93.09 ± 3.85^{cd}
T_{11} (AS+ CN+M)	197.39 ± 7.14^{c}	95.39 ± 3.69^{c}
T_{12} (UR+DCD)	201.07 ± 4.83^{bc}	114.01 ± 3.04^{ab}
T_{13} (UR+M)	224.29 ± 8.39^{ab}	110.90 ± 3.64^{b}
SE(d)	12.12	5.00
LSD(≤0.05)	25.01	10.32

Data represent the mean ± standard error of three independent determinates. Means within a column that did not differ significantly at 5% level of significance when compared with Fisher's Least Significant Difference are followed by the same superscript letters

Daily water use (L) = Evaporation (mm) × 0.7 × Canopy ground area (m^2)

Vegetative growth parameters measured before and at the end of experiment. At the initial and at the end of experiment, tree height, spread (N-S; E-W) and shoot growth rate (three uniform shoots per plant) were measured with the help of pre-marked bamboo pole of 10 m length and measuring tape. The per cent increase in length was calculated on the basis of initial and final length. The specific leaf area was calculated by dividing leaf area with dry weight. Physiological and biochemical parameters were recorded at 45 days after each fertilizer application. Photosynthetic and respiration rate and stomatal conductance were measured by using Infra-Red Gas Analyser PS System II (Li-Cor 6200). The leaf chlorophyll content (chlorophyll a, b and total) was estimated by following the method suggested by Hiscox and Israelstam (1979). Total sugars in leaf of each treatment were estimated by the anthrone method (Hodge and Hofreiter 1962). Soluble protein content of the samples were estimated according to method suggested by Lowry *et al*. (1951). Estimation of *in vivo* nitrate reductase activity was done by using the method of Klepper *et al*. (1971).

The data were statistically analysed for analysis of variance (ANOVA) using IASRI Server using SSCNARS portal. Means were separated using Fisher's least significant difference at 5 per cent level of significance. Grouping of letters on treatments were made using pdglm800.sas.

RESULTS AND DISCUSSION

Vegetative growth

At the end of experiment, different N-forms alone (without NI) were compared and found that all the growth parameters were higher in treatment containing urea and ammonium sulphate. When different forms of N-fertilizers were amended with DCD and meliacins than the percentage increase in tree height found significantly higher in treatment AS+DCD (44.05%) followed by on par with AS+meliacins, urea +meliacins and urea+DCD. Tree spread (E-W) found statistically highest in treatment T_7 (AS+meliacins; 77.33%); whereas, tree spread (N-S) also found maximum in treatment T_7 (66.03%) but remained statistically similar in treatment AS+meliacins (T_6), urea+meliacins (T_{13}) and urea+DCD (T_{12}). As far as specific leaf area is concerned it found statistically higher in treatment T_7 (123.86 cm^2/g) followed by T_6 and T_{13} (Table 1). Shoot growth rate was found maximum (247.39%) with the application of AS+meliacins (T_7) and found statistically equal to T_6 and T_{12}. All the growth parameters were found statistically lowest in control treatment (T_1).

The continuously higher soil inorganic N contents for the DCD and meliacins treatments were also beneficial for the growth and N assimilation of the Kinnow plants. Therefore, we observed higher plant height, spread and shoot growth rate for the DCD and meliacins treatments. Therios and Sakellariadis (1988) observed a greater vegetative growth of olive plants when they were fertilized with ammonium

Table 2 Effect of different nitrogen sources with or without nitrification inhibitors on chlorophyll a and chlorophyll b (mg/g Fw) content in Kinnow plants

Treatment	Chlorophyll a (mg/g Fw)			Chlorophyll b (mg/gFw)		
	1st split	2nd split	3rd split	1st split	2nd split	3rd split
T_1 (Control)	1.62±0.07^{f}	1.88±0.02^{f}	1.68±0.08^{f}	0.23±0.01^{f}	0.30±0.03^{c}	0.26±0.01^{d}
T_2 (AS)	2.34±0.05^{bcde}	2.43±0.05^{cde}	2.38±0.09^{bcd}	0.65±0.01^{d}	0.71±0.02^{b}	0.67±0.03^{c}
T_3 (CN)	2.23±0.06^{de}	2.29±0.10^{de}	2.23±0.08^{de}	0.62±0.03^{de}	0.67±0.03^{b}	0.65±0.03^{c}
T_4 (AS+ CN)	2.27±0.03^{cde}	2.33±0.06^{cde}	2.29±0.10^{cd}	0.61±0.02^{de}	0.69±0.02^{b}	0.64±0.03^{c}
T_5 (UR)	2.27±0.06^{cde}	2.41±0.11^{cde}	2.31±0.04^{cd}	0.64±0.03^{d}	0.67±0.03^{b}	0.67±0.03^{c}
T_6 (AS+DCD)	2.55±0.09^{a}	2.84±0.10^{a}	2.64±0.09^{a}	0.80±0.03^{a}	0.85±0.03^{a}	0.83±0.03^{a}
T_7 (AS+M)	2.45±0.06^{abc}	2.75±0.06^{ab}	2.61±0.06^{a}	0.79±0.02^{ab}	0.84±0.02^{a}	0.82±0.02^{a}
T_8 (CN+DCD)	2.19±0.05^{e}	2.19±0.05^{e}	2.03±0.05^{e}	0.64±0.02^{d}	0.66±0.02^{b}	0.67±0.02^{c}
T_9 (CN+M)	2.25±0.09^{de}	2.23±0.09^{e}	2.08±0.09^{e}	0.67±0.03^{cd}	0.71±0.03^{b}	0.70±0.03^{bc}
T_{10} (AS+CN+DCD)	2.39±0.01^{abcd}	2.55±0.09^{bc}	2.52±0.02^{ab}	0.61±0.01^{de}	0.72±0.03^{b}	0.64±0.01^{c}
T_{11} (AS+CN+M)	2.44±0.07^{abc}	2.52±0.09^{bcd}	2.50±0.09^{abc}	0.57±0.02^{e}	0.72±0.03^{b}	0.63±0.02^{c}
T_{12} (UR+DCD)	2.49±0.06^{ab}	2.77±0.06^{ab}	2.63±0.06^{a}	0.77±0.02^{ab}	0.82±0.02^{a}	0.79±0.02^{a}
T_{13} (UR+M)	2.46±0.05^{ab}	2.74±0.13^{ab}	2.60±0.06^{a}	0.73±0.03^{bc}	0.80±0.04^{a}	0.76±0.03^{ab}
SE(d)	0.09	0.12	0.10	0.03	0.04	0.04
LSD(≤0.05)	0.18	0.25	0.21	0.07	0.08	0.07

Data represent the mean ± standard error of three independent determinates. Means within a column that did not differ significantly at 5% level of significance when compared with Fisher's Least Significant Difference are followed by the same superscript letters.

Table 3 Physiological variables of Kinnow mandarin plant influenced by different nitrogen sources with or without nitrification inhibitors

Treatment	Photosynthesis rate (μmol/m^2/s)			Transpiration rate (mmol/m^2/s)			Stomatal conductance (mmol/m^2/s)		
	1st split	2nd split	3rd split	1st split	2nd split	3rd split	1st split	2nd split	3rd split
T_1 (Control)	3.48±0.14^{e}	3.88±0.16^{d}	3.08±0.14^{e}	1.60±0.07^{e}	1.61±0.14^{e}	1.48±0.07^{e}	21.65±0.88^{f}	22.08±1.97^{f}	21.43±0.96^{g}
T_2 (AS)	4.43±0.10^{bcd}	4.61±0.10^{c}	4.42±0.10^{bcd}	1.86±0.04^{d}	1.94±0.04^{cd}	1.82±0.07^{cd}	25.23±0.56^{de}	26.60±0.59^{e}	25.62±1.01^{ef}
T_3 (CN)	4.35±0.20^{cd}	4.42±0.20^{c}	4.40±0.20^{cd}	1.84±0.08^{d}	1.91±0.09^{cd}	1.80±0.08^{cd}	26.24±1.19^{de}	26.09±1.18^{e}	24.28±1.10^{fg}
T_4 (AS+ CN)	4.30±0.11^{d}	4.29±0.11^{cd}	4.35±0.11^{d}	1.85±0.05^{d}	1.85±0.05^{d}	1.77±0.08^{cd}	24.85±0.62^{e}	27.94±0.70^{de}	25.54±1.08^{ef}
T_5 (UR)	4.40±0.19^{bcd}	4.48±0.20^{c}	4.50±0.22^{bcd}	1.90±0.08^{cd}	1.84±0.08^{d}	1.80±0.09^{cd}	26.02±1.14^{de}	29.63±1.30^{cde}	28.27±1.35^{de}
T_6 (AS+DCD)	5.06±0.17^{a}	5.95±0.20^{a}	5.71±0.19^{a}	2.12±0.07^{ab}	2.35±0.08^{a}	2.07±0.07^{ab}	37.52±1.27^{a}	38.41±1.30^{a}	36.82±1.25^{a}
T_7 (AS+M)	5.00±0.12^{a}	5.75±0.13^{ab}	5.55±0.13^{a}	2.15±0.05^{a}	2.24±0.05^{ab}	2.13±0.05^{a}	37.39±0.86^{a}	37.12±0.86^{a}	35.76±0.82^{ab}
T_8 (CN+DCD)	4.29±0.10^{d}	4.52±0.11^{c}	4.33±0.11^{d}	1.88±0.05^{d}	1.86±0.05^{cd}	1.97±0.05^{abc}	28.00±0.68^{bcd}	29.05±0.70^{cde}	28.32±0.69^{de}
T_9 (CN+M)	4.40±0.18^{bcd}	4.44±0.18^{c}	4.42±0.20^{bcd}	1.86±0.08^{d}	1.87±0.08^{cd}	1.72±0.08^{d}	27.49±1.12^{cde}	31.36±1.28^{cd}	27.22±1.22^{def}
T_{10} (AS+CN+DCD)	4.80±0.02^{abc}	5.33±0.19^{b}	4.90±0.02^{b}	1.95±0.01^{bcd}	1.90±0.07^{cd}	1.94±0.01^{abc}	30.18±0.11^{bc}	32.33±1.15^{bc}	30.12±0.11^{cd}
T_{11} (AS+CN+M)	4.82±0.18^{ab}	5.43±0.20^{ab}	4.86±0.18^{bc}	1.90±0.07^{cd}	1.78±0.07^{de}	1.89±0.07^{bcd}	31.00±1.16^{b}	35.11±1.31^{ab}	32.88±1.23^{bc}
T_{12} (UR+DCD)	5.00±0.12^{a}	5.70±0.26^{ab}	5.50±0.13^{a}	2.08±0.05^{abc}	2.17±0.05^{ab}	2.07±0.05^{ab}	35.84±0.85^{a}	36.51±0.85^{a}	34.27±0.81^{ab}
T_{13} (UR+M)	4.95±0.23^{a}	5.66±0.13^{ab}	5.52±0.25^{a}	2.02±0.09^{abcd}	2.08±0.10^{bc}	2.10±0.10^{a}	36.04±1.64^{a}	35.76±1.66^{ab}	34.40±1.57^{ab}
SE(d)	0.22	0.25	0.24	0.09	0.11	0.10	1.47	1.75	1.56
LSD(≤0.05)	0.46	0.52	0.49	0.19	0.23	0.21	3.03	3.60	3.21

Data represent the mean ± standard error of three independent determinates. Means within a column that did not differ significantly at 5% level of significance when compared with Fisher's Least Significant Difference are followed by the same superscript letters

or urea than when nitrate was the N form of fertilization. The same observations were reported by Garcia *et al*. (1999) when a sterile sand substrate was used to grow olive plants. Colugnati *et al*. (1997) studied on grapevine and indicated in its results that nitrogen supply by isobutylidenediurea (IBDU) and dicyandiamide (DCD) positively influence total buds, sprouting buds and cluster numbers. These nitrogen strategies achieve more balanced plants.

Physiological processes

It is evident from data that different N-sources and nitrification inhibitors exerted significant variation on a, b and total chlorophyll content of leaves, photosynthetic rate, leaf transpiration rate and stomatal conductance of Kinnow during all three split application of fertilizers (Table 2, 3). In the present study, a, b and total chlorophyll content were found higher with aplication of AS and urea among the untreated N-fertilizers; but, addition of nitrification inhibitors (DCD and meliacins) outperformed. The application of AS+DCD registered significantly maximum values of a, b and total chlorophyll content (2.55, 2.84, 2.64; 0.80, 0.85, 0.83 and 3.35, 3.69, 3.47 during first, second and third split application, respectively). The use of ammonium fertilizers and the addition of nitrification inhibitors to ammonium fertilizers led to a greener leaf color, probably due to an increase of the chlorophyll content in NH_4^+-N fed plants (Bonasia *et al*. 2008). Nitrification inhibitors (NI) had positive response to photosynthetic rate at all three split fertilizer application. When different N-fertilizers treated with NI (DCD and meliacins) then treated ammonium sulphate (AS), urea (UR) and combination of AS+calcium nitrate (CN) exhibited statistically higher mean photosynthetic rate than untreated N-fertilizers. The value of mean photosynthetic rate at all three season was significantly higher with the treatment AS+DCD (5.06, 5.95 and 5.71 µmol/m^2/s with AS+DCD during first, second and third split application, respectively); however, it remained statistically similar to AS+meliacins, urea+meliacins and urea+DCD. Among the untreated N-fertilizers, AS followed by urea showed higher photosynthetic rate. The control exhibited the lowest photosynthetic rate.

The untreated N-fertilizers had comparatively lesser leaf transpiration rate than respective N-fertilizers treated with NI (DCD and meliacins) in all three split fertilizer application and lowest in control (Table 3). Application of AS+meliacins registered significantly maximum values of transpiration rate, i.e. 2.15 and 2.13 mmol/m^2/s during winter and summer split application respectively; whereas, in winter (second split) it was found higher under AS+DCD treatment (2.35 mmol/m^2/s). But both the treatments (AS+meliacins and AS+DCD) along with urea+DCD and urea+meliacins remained on par with respect to transpiration rate at all three seasons. Stomatal conductance showed the same trend as presented in the photosynthetic rate (Table 3). Like photosynthetic rate similarly, mean values of stomatal conductance in all three season was significantly higher with the treatment AS+DCD (37.52, 38.41 and 36.82 mmol/m^2/s), however, it remained statistically similar to AS+meliacins, urea+meliacins and UR+DCD. In other hardwood species, chlorophyll content (El Kohen and Mousseau 1994), net photosynthesis (Ibrahim *et al*. 1998, Kubiske *et al*. 1998), or both (Bondada and Syvertsen 2003) increased with N fertilization consistent with the results of our study as nitrification inhibitors reduce NO_3^- leaching (Dhakar *et al*. 2015) and to increase N uptake by trees.

Table 4 Effect of different nitrogen sources with or without nitrification inhibitors on nitrate reductase (NR) activity in leaves of Kinnow mandarin

Treatment	NR (µmol nitrite formed/g FW/h)		
	Autumn	Spring	Summer
T_1 (control)	212.00±7.29^{f}	206.52±7.10^{g}	200.98±6.91^{d}
T_2 (AS)	497.02±19.10^{a}	478.31±18.38^{a}	469.68±18.05^{a}
T_3 (CN)	314.07±7.10^{de}	276.64±12.59^{f}	309.98±17.07^{c}
T_4 (AS+ CN)	376.58±15.98^{b}	383.60±16.28^{bc}	324.44±13.77^{c}
T_5 (UR)	353.71±12.75^{bcd}	389.96±14.06^{b}	408.95±14.75^{b}
T_6 (AS+DCD)	368.95±13.13^{b}	372.65±12.62^{bcd}	375.34±12.71^{b}
T_7 (AS+M)	360.20±12.75^{bc}	361.54±12.79^{bcde}	369.57±13.08^{b}
T_8 (CN+DCD)	311.20±11.51^{e}	261.14±9.66^{f}	319.29±11.81^{c}
T_9 (CN+M)	323.70±13.20^{cde}	278.16±11.34^{f}	325.71±13.28^{c}
T_{10}(AS+CN+DCD)	350.20±18.54^{bcde}	345.31±18.28^{cde}	383.47±20.30^{b}
T_{11}(AS+CN+M)	357.60±13.38^{bc}	352.61±13.19^{bcde}	381.92±14.29^{b}
T_{12} (UR+DCD)	328.40±13.42^{cde}	326.82±13.35^{e}	306.40±12.52^{c}
T_{13} (UR+M)	340.70±13.09^{bcde}	333.92±12.83^{de}	319.92±12.30^{c}
SEd±	19.32	19.04	20.45
LSD (≤0.05)	39.88	40.04	42.22

Biochemical constituents

All the N-sources with and without nitrification inhibitors produced significantly higher total soluble sugars and proteins in leaves of Kinnow over control. Among different N-fertilizers, ammonium sulphate treated plants showed higher total soluble sugar and proteins. However, ammonium sulphate treated with DCD produced statistically highest total soluble sugar (9.22, 9.78 and 9.40% leaf fresh wt) and soluble proteins (74.80, 76.49 and 71.96 mg/g leaf dry wt) during winter, autumn and summer, respectively followed by ammonium sulphate treated with meliacins. Horchani *et al*. (2010) also found increase in carbohydrate concentration in plants under NH_4^+-N fertilization in comparison to NO_3^--N.

Nitrification inhibitors slowdown the conversion of ammonia to nitrate so, the ammonium sulphate and urea treated with nitrification inhibitors showed lower nitrate reductase activity due to lower substrate (NO_3^-) availability than untreated both the fertilizers (Table 4). Nitrification of ammonium sulphate resulted into the availability of NO_3^- ion in the soil. So, application of ammonium sulphate registered maximum values of nitrate reductase activity, i.e. 497.02, 478.31 and 469.68 (µmol nitrite formed/g f wt) during winter, autumn and summer, respectively due to higher substrate (NO_3^-) availability under this treatment. Frith (1972) in his study also found that apple plants grown in ammonium nitrate solutions had lower nitrate-reductase activity than those grown in other nitrate solutions.

The sources of N have a strong impact on growth and physio-biochemical parameters in Kinnow plants. Nitrogen as ammonium sulphate is less susceptible to leaching and more efficient in sandy loam soil with high pH conditions. Additionally, higher soil inorganic N contents for the DCD and meliacins treatments were also beneficial for the growth and provide more appropriate N to support growth and retain more N in the leaves to maintain the photosynthetic apparatus in Kinnow mandarin for better plant health and performance.

ACKNOWLEDGEMENT

Authors are grateful to Department of Science and Technology, New Delhi, for Senior Research Fellowship to the first author.

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