नत्रजन स्त्रोतों एवं नाइट्रीकरण संदमकों का नत्रजन-गतिशीलता और किन्नों पादप प्रदर्शन पर प्रभाव

EFFECT OF NITROGEN SOURCES AND NITRIFICATION INHIBITORS ON NITROGEN DYNAMICS AND PLANT PERFORMANCE OF KINNOW MANDARIN

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Effect of Nitrogen Sources and Nitrification Inhibitors on Nitrogen Dynamics and Plant Performance of Kinnow Mandarin

By

Mahesh Kumar Dhakar

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This is to certify that the thesis entitled **"Effect of nitrogen sources and nitrification inhibitors on nitrogen dynamics and plant performance of Kinnow mandarin"** submitted to the Post-Graduate School, Indian Agricultural Research Institute, New Delhi, India in partial fulfillment of the requirements for the award of **Doctor of Philosophy** degree in **Horticulture** embodies the results of a *bona fide* research work carried out by **Mr. Mahesh Kumar Dhakar, Roll No. 9848** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

It is further certified that all the assistance and help availed during the course of investigation as well as all sources of information have been duly acknowledged by him.

Date: 29/12/2014
Place: New Delhi

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Abbreviations

°C	:	degree Celsius
%	:	per cent
cm	:	centimetre
µmol m^{-2} s^{-1}	:	micro moles per metre square per second
d.f.	:	Degrees of freedom
EC	:	Electrical conductivity
A	:	absorbance
LSD	:	Least significant difference
ANOVA	:	analysis of variance
AR	:	analytical reagent
et al.	:	(*et aliia*) and other
etc.	:	(*et cetera*) and the rest
Fig.	:	Figure
g	:	Gram
Ha	:	Hectare
h.	:	Hour(s)
i.e.	:	(*id est*) that is
K	:	Potassium
m^2	:	Square meter
Max.	:	Maximum
HCl	:	hydrochloric acid
mg	:	milligram
Min.	:	Minimum
mm	:	millimetre
N	:	Nitrogen
NS	:	Non-significant
P	:	Phosphorus
l	:	litre
RBD	:	Randomized block design
min	:	minute(s)
ml	:	millilitre
mm	:	millimeter
viz.	:	(*videlicet*) namely
OD	:	optical density
v/v	:	volume/volume
w/v	:	weight/volume
l^{-1}	:	per litre
SEd±	:	Standard error of deviation
SEm±	:	Standard error of mean

1. Introduction

India ranks sixth among the major citrus producing countries of the world. Citrus is third important fruit after banana and mango in India with an area of 1.04 million hectares and production of 10.09 million MT of fruits (Anonymous, 2013). Among the citrus groups, Kinnow mandarin occupies a predominant place in the Indian citrus industry. Kinnow originated as a hybrid between King and Willow Leaf mandarin (*Citrus nobilis* x *C. deliciosa*) at UC Riverside, California. It was developed by Dr H.B. Frost in 1915 and was released for commercial cultivation in USA in 1935. In India, Kinnow was introduced in Punjab and has acclimatized well in the state and adjoining parts of Haryana and Rajasthan, beside Nagpur and Akola regions of Maharashtra. The factors which contributed to the success of this fruit is its attractive golden yellow fruit colour, size, good eating quality, deep yellowish-orange flesh colour, juicy, distinctive flavour, higher yield and juice content.

In India, nitrogenous fertilizers are used in large quantity in agriculture. The consumption of nitrogenous fertilizers during the year 2000-2001 was 10.92 million tonnes, whereas, it rose to 16.82 million tonnes during 2012-13 (Anonymous, 2014). This indicates that the consumption of nitrogenous fertilizers has increased by 6.38 million tonnes in the last eleven years and it is increasing further.

Citrus trees are moderate feeder of mineral nutrients for their growth and productivity. Nitrogen fertilization is generally required in greater amounts than any other plant nutrients to maximize tree growth and development. Nitrogen has numerous functions in plants and essentially all life processes depend on it. An abundant supply of N compounds is required in each plant cell for normal cell division, growth and respiration. Even the green leaf pigment chlorophyll which enables plants to use the energy of sunlight to form sugars from carbon dioxide and water, is a nitrogenous compound. Citrus absorb nitrogen either as nitrate or in ammonical forms. Nitrate has the advantage of immediate availability to plants and microbes, but it has disadvantages of high solubility and mobility in the soil. In contrast to nitrate, ammonium is not subjected to losses because it can be held by soil clay minerals. Ammonium pools are

always larger in the top 10 cm of soil, but NO_3^- fluctuates throughout the year in soil profile and always depletes during periods of rapid plant growth (Jackson *et al*., 1988). Some plants grown in nutrient solution under controlled conditions absorb nitrate more readily than ammonium ion while other plants prefer ammonium. In several crops, combination of NH_4^+ and NO_3^- usually results in greater vegetative growth than either of the N form alone.

Less than 20 per cent of the nitrogen applied to orchards seems to be recovered by the fruit trees (Miller and Smith, 1976). Recovery of N from nitrogenous fertilizers applied to soil is low due to the loss of N in the form of leaching, run-off and gaseous emissions. Nitrogen uptake efficiency (NUE), defined as the percentage of applied N taken up by crops, is often low in soils because of the high mobility of N fertilizer (Obreza and Rouse, 1992). In sandy soils receiving 100 to 125 cm of rainfall, the efficiency of N uptake by plants may not exceed 20 to 30% (Oertli and Lunt, 1962). Paramasivam *et al*. (2001) showed that NUE for 25-year-old 'Hamlin' orange trees grown in an Entisol ranged between 40 and 53%. Low nutrient use efficiency causes a negative impact on the environment and contributes to groundwater pollution. Currently, there is an increasing concern regarding large accumulations of NO_3-N in ecosystems, mainly from N leaching from agricultural fields. Excess of nitrate not used by trees is subject to leaching losses below the rooting zone in citrus (McNeal *et al*., 1995). This downward movement reduces the efficiency of applied N-fertilizer and may cause the contamination of ground water. Water quality concerns have been raised in the Florida citrus industry, because of increasing levels of nitrate concentration in groundwater near major citrus producing regions (Graham and Alva, 1996).

High nitrate concentrations are related to methemoglobinemia in infants and ruminants; stomach cancer for which a possible link with nitrates or nitrosoamines has been suggested; other diseases such as goiter, birth defects, and heart diseases; and eutrophication of surface water (Shaviv and Mikkelsen, 1993). Most public health authorities agree that the maximum allowable nitrate concentration for potable water is 10 ppm NO_3^--N. Water containing excessive nitrate can cause methemoglobinemia, the oxidation of ferrous to ferric hemoglobin, rendering the blood unable to transport oxygen to tissue. Human infants and polygastric animals seem to be most susceptible to

methemoglobinemia. Ridder and Ochme (1974) attributed consumption of nitrate laden water as causing "chronic nitrate toxicity", responsible for a multitude of disorders.

To control gaseous emissions from fertilizer N, nitrate leaching and to obtain high N use efficiency by crop plants, nitrification inhibition is thus desirable. Nitrification is the biochemical oxidation of ammonium to nitrate. *Nitrosomonas* and *Nitrobacter* bacterial species are involved in nitrification in soil. *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 (Subbarao *et al*., 2006). A number of chemicals have been used to inhibit nitrification in soil. These include 2-chloro-6 (trichloromethyl) pyridine (nitrapyrin), sulfathiazole, dicyandiamide, 2-amino-4-chloro-6-methyl pyrimidine, 2-mercaptobenzothiazole, thiourea and 5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole (terrazole), 3,4-dimethyl pyrazol phosphate (DMPP) etc. Nitrapyrin, dicyandiamide (DCD) and acetylene are 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, 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. However, 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.

Management of nitrification in soil by nitrification inhibitors is a proven strategy for improving N-recovery, agronomic N-use efficiency (NUE) and at the same time limiting environmental pollution. However, little information is available on Kinnow mandarin response to the different nitrogen sources and nitrification inhibitors. Keeping the above facts in view, the present experiment was carried out with the following objectives:

1. To find out effect of different nitrogen sources and nitrification inhibitors on nitrogen dynamics in soil under Kinnow plants.
2. To determine effect of different nitrogen sources and nitrification inhibitors on vegetative growth, physiological processes and biochemical constituents of Kinnow plants.
3. To find out changes in soil biological activities due to nitrogen sources and nitrification inhibitors.

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2. Review of Literature

Nutrient availability is a limiting factor in citrus cultivation in dry tropical and sub-tropical regions of the world which leads to low soil fertility in general. Producers require application of chemical fertilizers to improve soil productivity. Citrus is an exhaustive crop and inadequate plant nutrition cause serious disorders in citrus and may eventually lead to decline of the orchards. Fertilizer alone on an average, constitutes about 20-30% of total cost of citrus production, which is a significant recurring expenditure, a grower needs to invest every year (Srivastava *et al.*, 2008). Nitrogen is the most important nutrient, which is required for proper growth and development of the plants and citrus including Kinnow is not exception for that. Therefore, it is important to increase the nitrogen use efficiency by using nitrification inhibitors.

Keeping these points in view, the literature pertaining to effect of nitrogen sources and nitrification inhibitors on nitrogen dynamics in soil, leaf nutrient acquisition, soil biological, growth, physiological and biochemical parameters of citrus is reviewed and presented in the following sections.

2.1 Nitrogen dynamics in soil

Nitrogen is the main and most frequently yield-limiting nutrient for growth and yields of most fruit crops. The nitrogen use efficiency of various nitrogenous fertilizers is very low because of losses by various processes like ammonia volatilization, leaching and nitrification-denitrification. Nitrogen fertilizer applied in excess of crop requirements can result in accumulation of nitrate-N (NO_3^--N) in the soil profile (Guillard *et al*., 1995; Malhi *et al*., 2002).

The source of nitrogen applied to the soil can significantly affect the rate of nitrate leaching. When the nitrogen source is in the ammonical form, resistance to leaching occurs due to cationic attraction of ammonium ions by clay and humus. Nitrate ions are highly mobile in the soil, contributing to the contamination of ground waters, can suffer denitrification and accumulate in plant tissues, whereas, the ammonium ion is not as readily subject to leaching loss. Wendt *et al*. (1976) stated that no one source of fertilizer

reduced long term nitrate leaching. However, the ammoniacal forms of nitrogen should maintain the nitrogen in the root zone for a longer period, allowing an increased opportunity for plant uptake. The efficiency of urea is decreased by losses of N as ammonia gas after the urea is hydrolyzed at the soil surface by reaction with the enzyme urease. Ammonia loss from ammonium nitrate or calcium ammonium nitrate is low.

Nitrification converts a relatively immobile NH_4^+-nitrogen (N) to highly mobile NO_3^--N, and this has implications not only for N-use efficiency for crop production but also for environmental quality (Sahrawat, 1989; Subbarao *et al*., 2006). Most public health authorities agree that the maximum permissible nitrate concentration for potable water is 10 ppm NO_3^- -N.

Nitrogen fertilization is one of the primary practices in citrus production, and growers widely use NH_4^+ salts as N sources. The NH_4^+ released by solubilization of these N fertilizers in the soil water is rapidly transformed into NO_3^- by nitrifying bacteria. Then, a part of the NO_3^- produced is mainly lost through leaching. This loss contributes to NO_3^- pollution of groundwater and also accounts for the low efficiency of the N fertilization in citrus (Feigenbaum *et al*., 1987).

Wallace (1954) examined the uptake of ^{15}N-labeled ammonium and nitrate by citrus trees and found that in water culture, Valencia orange cuttings absorbed more ammonium than nitrate. The reports from Smith (Smith, 1957; Smith and Rasmussen, 1957) showed that citrus seedlings grew normally with exclusive NH_4^+-N as a source provided excess acidity is avoided. Latter, Smith and Rasmussen (1961) concluded that citrus can be grown on a wide range of N sources with equal success. Urea is the most common N fertilizer used for various crops. Gaseous loss of ammonia following urea fertilizer application may account for up to 75% of total N applied (Fenn and Miyamoto, 1981), whereas, N volatilization from other forms of nitrogen fertilizer is less, especially in acid soils (Havlin *et al*., 1999). Mattos (2000) estimated NUE for 6-year-old 'Valencia' trees grown in a sandy soil to be 40 and 26% for ammonium nitrate and urea, respectively. Paramasivam *et al*. (2001) showed that NUE for 25-year-old 'Hamlin' orange trees grown in an Entisol ranged between 40 and 53%. In a study related to effects of nitrogen fertilization on N losses and fruit yield of 6-yr-old 'Valencia' sweet

orange, Cantarella *et al*. (2003) found that ammonia volatilization reached 26 to 44% of the applied urea at the highest doses. Ammonia volatilization losses with ammonium nitrate were lower (4% of the N applied). Since less N was lost from ammonium nitrate, it may be a preferred N source for broadcast application when NH_4^+ volatilization is a concern.

The improvement of the fertilizer N use efficiency is necessary for a sustainable agriculture. There are several methods of reducing N loss and improving N-use efficiency of crops. Such methods include proper time and methods of application of fertilizers, use of slow release fertilizers and use of nitrification inhibitors.

In this direction, the use of specific nitrification inhibitors may increase the N fertilizer uptake and decrease nitrate leaching. Previous studies demonstrated that the nitrification inhibitor (NI) dicyandiamide (DCD) added to ammonium sulphate nitrate (ASN) improved the N-fertilizer efficiency and reduced nitrate leaching in young and mature citrus trees (Serna *et al*., 1994, 1996). Raigon *et al.* (1999) compared the amounts of available N in a soil cylinder corresponding to the drip area of the orange tree and of 0–60 cm depth at different vegetative periods in an orange orchard and found that sulphur-coated urea (SCU) treatment applied in low doses and only in spring maintained high levels of available N in soil during the vegetative cycle, reducing N losses, compared with ammonium nitrate sulphate (ANS) treatments. Preliminary studies carried out in young citrus trees grown in soil culture in pots, revealed a remarkable effect of DMPP on decreasing NO_3^--N levels both in soil and in leaching water, as well as an increase in N uptake of treated plants (Serna *et al*., 2000). Banuls *et al*. (2001) evaluated the inhibitory action on nitrification of 3,4-dimethylpyrazole phosphate (DMPP) added to ammonium sulfate (AS) and applied as a fertilizer to citrus plants and its effects on mineral-N in the soil. They reported that the addition of DMPP to AS resulted in higher levels of NH_4^+-N and lower levels of NO_3^--N in the soil during the whole experimental period. The NO_3^--N concentration in drainage water was lower in the AS plus DMPP- treated pots. The results indicate that the DMPP nitrification inhibitor improved N fertilizer efficiency and reduced nitrate-leaching losses by retaining the applied N in the ammoniacal form. Similar results were also found by Martinez-Alcantara *et al*. (2013) during their experiment on citrus. Di and Cameron

(2002, 2007) demonstrated that using the nitrification inhibitors such as DCD significantly decreased nitrate leaching and N_2O emissions in grazed pastures in New Zealand. Quinones *et al*. (2009) carried out an experiment with Clementine cv. Nules grafted on Troyer citrange (*Citrus sinensis* x *Poncirus trifoliata*) rootstock under field conditions and found that the NH_4^+-N concentration in the 0-20 and 20-40 cm soil layers was significantly higher in the ammonium sulphate (AS) + nitrification inhibitor (NI) treatment. In contrast, the NO_3^--N concentration was significantly higher in the soil treated only with AS. Ge *et al*. (2011) conducted a field experiment to investigate the effect of dicyandiamide (DCD) and DCD + S on nitrification-inhibiting on apple orchard soil. The results were as follows: soil NH_4^+-N contents in N + DCD and N + DCD + S treatments were higher than that of N treatment within 40 days after fertilization. Soil NO_3^--N content was obviously lower in the two nitrification inhibitor treatments than that of other N treatments. Compared with N treatment, gross nitrification and denitrification rates in soil were low in N + DCD and N + DCD + S treatments, and the biggest difference appeared at early times.

Neem (*Azadirachta indica*) cake was found to inhibit nitrification effectively both in the laboratory and green-house conditions (Sahrawat and Parmar, 1975). According to Kumar *et al.* (2007), the per cent nitrification inhibition (NI) of all of the neem oils (25 samples of neem oils having diverse backgrounds in terms of ecotype, method of preparation, age of the sample etc.) caused NI ranging from 4.0 to 30.9% in a soil incubation experiment. They also found that the meliacins content in neem oil directly affected the nitrification inhibition.

Majumdar *et al*. (2002) studied the effect of four nitrification inhibitors [crushed neem seed powder coated urea; nimin (commercial derivative of neem) coated urea; dicyandiamide (DCD) and thiosulfate] with urea in wheat crop and found that nimin coated urea inhibited nitrification the most, followed by urea + DCD, urea + thiosulfate and neem coated urea. Kumar *et al*. (2003) in an incubation experiment studied the comparative efficacy of organic isolates from non-edible oilseeds cakes (neem, sal, kusum and undi cakes with 15 and 30% level of applied urea) on nitrification of applied urea. Amongst the cakes, neem cake isolate was most effective in inhibiting nitrification process as compared to isolates of other cakes and maintained comparative higher

content of ammonical nitrogen (16.80 and 20.50 mg kg-1 NH_4^+-N) at 7th week in both the levels due to neem seed cake isolates as against 11.20 mg kg^{-1} in control.

Abbasi *et al*. (2011) conducted a laboratory experiment to examine the effects of nitrification inhibitors neem seedcake (*Azadirachta indica*) (NSC), sodium thiosulphate ($Na_2S_2O_3$) and calcium chloride ($CaCl_2$) on changes in NH_4^+-N (0-15 cm depth), inhibition of nitrification and recovery of applied nitrogen as 200 mg N kg^{-1} through urea in soil. Results indicated that addition of nitrification inhibitors NSC, $Na_2S_2O_3$, and $CaCl_2$ resulted in a decrease in the extent of NH_4^+ disappearance by 35, 44 and 30% and inhibited nitrification by 54, 64, and 59%, respectively. Apparent N recovery (ANR) in the treatment receiving UN alone was 63% that substantially increased to 83%, 89% and 76% in the treatments receiving UN+NSC, UN+$Na_2S_2O_3$, and UN + $CaCl_2$, respectively indicating 32, 41 and 20% increase in N recovery. Kumar *et al.* (2011) suggested that the coating of prilled urea with meliacins proved to be most effective in enhancing yield and NUE of rice as compared to uncoated prilled urea or coated with other neem oil components.

2.2 Effect of different nitrogen sources and nitrification inhibitors

2.2.1 Growth parameters

Nitrogen is necessarily needed for optimum vegetative, as well as reproductive growth (Alva *et al*., 2006). Plants absorb nitrogen either as nitrate or as ammonium ions. Numerous studies have shown that ammonical-N as a sole source of N is deleterious to the growth of many higher plants. The addition of nitrate however, alleviated the inhibitory effects of NH_4^+ on growth. In several crops, combinations of ammonical and nitrate N usually result in greater vegetative growth than when either N form is used alone. In short term water culture experiment by Serna *et al*. (1992) with different ^{15}N labeled ammonium or nitrate concentrations, citrus seedlings absorbed NH_4^+ at a higher rate than NO_3^-. Although citrus trees absorb more ammonium than nitrate in short term water culture experiment, the greater uptake of ammonium does not always result in a better growth. In both water culture (Yokomizo and Ishihara, 1973) and sand culture (Yokomizo, 1975) nitrate nutrition was better than ammonium nutrition for citrus tree growth.

Dutra *et al*. (1994) found 10% more leaf surface, 6% more graft diameter, 5% more graft length and 7.6% more spring knots in controlled-release fertilized Carrizo citrange seedlings (*Citrus sinensis* x *Poncirus trifoliata*) grafted with Hernandina tangerine (*Citrus reticulata* Blanco), compared to typical fertigation (calcium nitrate, potassium nitrate, monoammonium phosphate and micronutrients). Colugnati *et al*. (1997) studied grapevine and indicated in results that nitrogen supply by isobutylidene diurea (IBDU) and dicyandiamide (DCD) positively influence total buds, sprouting buds and cluster numbers. These nitrogen strategies achieved more nutrient balanced plants. Martinez-Alcantara *et al*. (2013) observed increased biomass with a more profuse development of root system in citrus trees fertilized with AS + DMPP.

The highest plant height of rice was observed in meliacins-coated urea, which was significantly higher than other neem oil components' coated urea (Kumar *et al*., 2011).

2.2.2 Physiological and Biochemical parameters

Martin (1940) reported that the accumulation of carbohydrates in the leaves and twigs of grapefruit was reduced by a deficiency of nitrogen. Paul and Driscoll (1997) described the role of carbohydrates in signaling N deficiency through source:sink imbalance and showed that chlorosis is developed following the removal of the sink, causing depletion in total N, proline, arginine and serine. Asparagine is the predominant constituent of free amino acids found in young fruits of *Citrus unshiu* that showed a direct relation with leaf N status (Kato, 1983). Labanauskas and Handy (1971) observed that free amino acids, namely, proline, serine, alanine, and aspartic acid, were more prevalent in lemon (*Citrus limon* Burm.) and grapefruit (*Citrus paradisi* Macf.) leaves, together constituting as much as 77-79 per cent compared with 69 per cent of total free amino acids in Valencia orange [*Citrus sinensis* (L.) Osbeck], respectively. Changes in free amino acids have shown a greater promise than total N in understanding the N metabolism in citrus (Calot *et al*., 1988). Roots of ammonium compared to nitrate fertilized plants possessed higher sugar levels (Martins-Loucao *et al*., 2000). Thus, ammonium-N fertilized plants may differ from nitrate supplied plants with respect to passive sugar efflux from roots. Singh (1996) studied the effect of bio- and chemical-

fertilizers on chlorophyll content in sweet orange cv. Mosambi and found that total chlorophyll content of sweet orange leaves was positively correlated with nitrogen uptake.

Glutamine synthetase (GS, EC 6.3.1.2) in conjunction with glutamate synthase (GOGAT, EC 1.4.1.13) enzymes are responsible for the most important route of NH_4^+ assimilation in higher plants (Miflin and Lea, 1977). The central function of the GS/GOGAT cycle in avoiding excess levels of endogenous ammonium has been confirmed. Large numbers of biochemical and molecular biological studies (Lancien *et al*., 2000; Inokuchi *et al*., 2002) have established that many enzymes including nitrate reductase, nitrite reductase, glutamine synthetase, glutamate synthase, glutamate dehydrogenase, aspartate aminotransferase, aspergine synthase, and phosphoenol pyruvate carboxylase are involved in nitrogen assimilation. These enzymes exist in multiple forms in different sub-cellular compartments within different organs and tissues. Singh (1996) studied nitrate reductase (NR) activity in sweet orange cv. Mosambi and found that NR activity was correlated with the amount of nitrogen fertilizer. A number of studies (Trapaidze, 1986; Lawlor, 1994) have shown a close relationship between the activity of nitrate reductase, glutamate dehydrogenase, and total N in Washington Navel orange.

The presence of ammonium may reduce nitrate uptake. In citrus trees, this effect is suggested by the data of Wallace and Mueller (1957), but this phenomenon has not been directly examined. Apple trees showed a definite reduction of nitrate uptake in the presence of ammonium ion (Grasmanis and Nicholas, 1966, 1971; Fukumoto and Nagai, 1981). This reduced uptake of nitrate could be due to inhibition of nitrate and / or nitrate reductase activity by ammonium (Frith, 1972; Frith and Nicholas, 1975). Ramamurthy and Lodders (1982) observed that Calamondin (*Citrus madurensis* Lour.) trees showed a higher activity of glutamine synthetase treated with ammonium salts than trees treated with nitrate salts.

2.2.3 Leaf nutrient acquisition

Leaf tissue testing is a valuable tool to examine the tree nutritional status, particularly with respect to mobile nutrients such as N and K, and for micro-nutrients

such as copper, iron, manganese and zinc (Obreza *et al*., 1992). Plant tissue testing has been used on a wide number of crops as an indicator of soil and crop nutrient status, thereby, this information is used in fertilizer management decisions (Mills and Benton-Jones, 1996).

In comparison with the tissues of nitrate fed plants, the tissues of ammonium fed plants generally contain lower concentrations of inorganic cations (e.g. calcium, magnesium and potassium), higher concentration of the elements absorbed as anions (e.g. sulfur, phosphorous and chlorine), higher concentrations of amino compounds and lower concentrations of organic acids (Kirkby, 1981). These differences in physiological response to nitrogen sources may be related to pH regulation in tissues (Raven and Smith, 1976) and to differences in the metabolic processes of nitrate reduction and ammonium assimilation.

Serna *et al*. (1992) found a higher foliar N, P, Mg, Fe and Cu concentration in citrus fed with ammonium in comparison to nitrate. In the different ratios of ammonium and nitrate, leaf nutrients such as P, Mg, Fe, and Cu increased, whereas, Ca, K, Mn and Zn decreased with increasing proportions of ammonium in the N supply. The addition of DCD to ammonium sulphate nitrate resulted in a significant increase in leaf N concentration (Serna *et al*., 1994, 1996). Banuls *et al*. (2001) evaluate the inhibitory action on nitrification of 3,4-dimethylpyrazole phosphate (DMPP) added to ammonium sulfate (AS) applied as a fertilizer to citrus plants and its effects on nitrogen uptake. Nitrogen concentration in leaves and roots was higher with the application of AS + DMPP than with AS alone. The decrease in NO_3^- leaching as a result of DCD application also led to reduced leaching losses of cations, *e.g*. Ca_2^+, K^+ and Mg_2^+, due to reduced requirement of counter ions for NO_3^- (Di and Cameron, 2004b). Quinones *et al*. (2009) carried out an experiment with clementine cv. Nules grafted on Troyer citrange (*Citrus sinensis* x *Poncirus trifoliata*) rootstock under field conditions and found that the addition of NI to AS resulted in significantly higher N and Fe concentrations in the spring-flush leaves.

The higher concentration of N in grain and straw of rice coupled with higher dry matter yields resulted in higher total N uptake with meliacins-coated urea. In general,

meliacins-coated urea recorded the highest agronomic efficiency (AE) and apparent N recovery (ANR) than other components'-coated urea (Kumar *et al*., 2011).

2.3 Soil biological parameters

Microbial preference for NH_4^+ over NO_3^- has been reported by some researchers (Jansson, 1958; Azam *et al*., 1993). Ammonium has been found to be the preferred form of N for assimilation by microbes in many cultivated soils (Azam *et al*., 1993). Higher root and rhizosphere respiration with ammonium than nitrate nutrition has been ascribed to increased root exudation resulting in stimulation of bacterial growth (Trolldenier and Von Rheinbaban, 1981). The increased substrate availability in the rhizosphere of plants grown on ammonium instead of nitrate may also have significant bearing on the root colonization and activity of the beneficial microorganisms as well as of root pathogens. Studies with hydroponics suggested that ammonium compared to nitrate nutrition of plants increased microbial activity in the rhizosphere and that this stimulatory effect is attributable mainly to the increased C availability in the rhizosphere as the result of enhanced root exudation. While both NH_4^+ and NO_3^- are assimilated by microorganisms, the former is more readily assimilable not only because of its reported preference by the microbes (Jansson, 1958) but also because all the microbes may not necessarily synthesize nitrate reductase to enable them to assimilate NO_3^- (Azam *et al*., 1993). Hence, microbial proliferation in the rhizosphere and synthesis of aggregation enhancing macromolecules will be fairly dependent on the form of available N, while amount of soil adhering to the roots may be a reflection of the root exudation and microbial activity. The water concentration of root-adhering sand was found to be higher in the presence of NH_4^+ suggesting a higher rhizodeposition and/or microbial synthesis of polysaccharides that were assumed to be released from the roots or synthesized by rhizospheric microorganisms (Gill *et al.,* 2007). Mahmood *et al.* (2005) found that ammonium compared to nitrate nutrition of wheat and maize had significantly increased microbial biomass in the rhizosphere soil. In both plant species, ammonium vs. nitrate nutrition also increased aerobic and anaerobic respiration and dehydrogenase activity in the rhizosphere. They used dicyandiamide as nitrification inhibitor to maintain ammonium as the predominant N source for plants grown in ammonium treated soil.

Dicyandiamide (DCD) has a bacteriostatic effect on *Nitrosomonas* and the activities of other soil microorganisms are not influenced by DCD (Amberger, 1983; Di and Cameron, 2004a). DCD is well soluble in water and has to apply in high concentrations, but it is of low toxicity. In addition, DCD consists of 67% nitrogen and hence is also a slow release nitrogen fertiliser (Amberger, 1983). After repeated application on the same site, DCD has been observed to lose in efficacy. Because the soil microflora adapts to the active ingredient the mineralisation of DCD is accelerated (Rajbanshi *et al*., 1992). DCD is bacteriostatic rather than bacteriocidal and impairs the activity of ammonium-oxidising bacteria by restricting the uptake or utilization of ammonium (Zacherl and Amberger, 1990), thus reducing the production of NO_2^-. Various formulations of DCD have been developed and their efficacy has been measured under a range of environmental and soil conditions (Di *et al*., 2007; Kelliher *et al*., 2008; Monaghan *et al*., 2009). Since, it is readily water soluble it has little or no losses through volatility and is reasonably priced, DCD is the most used nitrification inhibitor in New Zealand (Zaman *et al*., 2009). Carneiro *et al.* (2010) found that dicyandiamide decreased microbial populations and activity, but did not alter composition. Among the three tested nitrification inhibitors [3,4-dimethylpyrazole phosphate (DMPP), 4-chloromethylpyrazole (ClMP), and dicyandiamide (DCD)], Tindaon *et al.*(2012) found that DCD had the smallest effect on dehydrogenase.

Edmeades (2004) suggested that research is needed to quantify the long- and short-term impacts of these chemicals, and their repeated use, on soil quality, which includes soil microbial function. In the face of growing evidence that changes in microbial community structure may lead to changes in soil microbial function, the effects of nitrification inhibitors on the soil microbial community must be considered.

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3. Materials and Methods

The field experiments were conducted at the Todapur Research Orchard, Indian Agricultural Research Institute, New Delhi, during winter, autumn and summer seasons of 2011-12 to study the "Effect of nitrogen sources and nitrification inhibitors on nitrogen dynamics and plant performance of Kinnow mandarin". This chapter provides the experimental details of materials used, techniques employed and observations taken during the course of investigation.

3.1 Description of the experimental site

The field experiment with Kinnow crop was carried out at the Todapur Orchard of Division of Fruits and Horticultural Technology, Indian Agricultural Research Institute, New Delhi. The experimental site is situated in West Delhi between the latitudes of 28^0 38' 22" N and 38^0 39' 05" N and longitudes of 77^0 9' 45" E and 77^0 10' 24"E at an average elevation of 228.61 m above the mean sea level. A map of IARI, New Delhi showing the location of experimental site at Todapur Orchard is shown in Fig. 3.1.

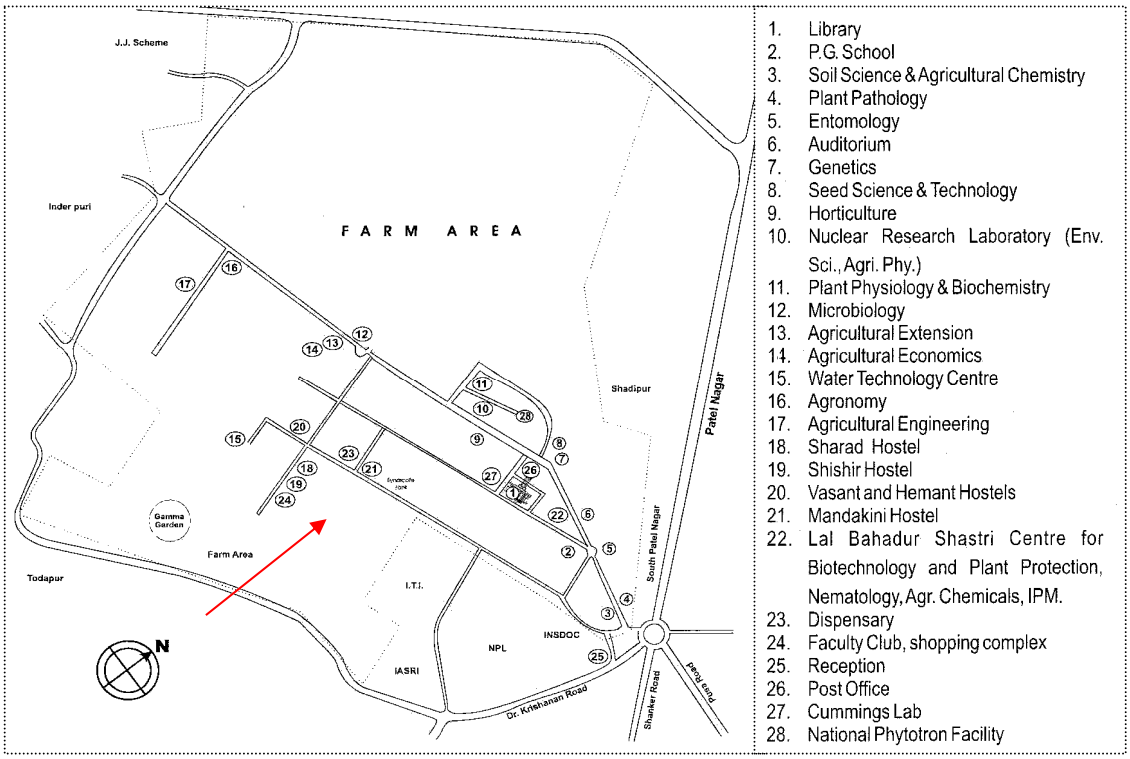

Fig. 3.1 Location Map of IARI and Todapur Orchard

3.2 Climate and weather

Climate of Delhi is categorized as semi-arid, sub-tropical with hot dry summer and cold winter and it falls in the Agro-eco-region-IV. The mean annual temperature is 25^0C. May and June are the hottest months with maximum temperature ranging from 40 to 45^0C. December and January are the coldest months with a temperature at 7^0C however, the minimum temperature dips to as low as 1^0C. The mean annual rainfall is 710 mm of which as much as 75 per cent is received during monsoon season (June to September). Some winter showers are also received during December- March. Frost occurs occasionally during month of December-January. The average relative humidity ranges from 34.1 to 97.9 per cent and average wind speed is 0.45 to 3.96 m per sec. The meteorological data collected from Agromet Observatory, Division of Agricultural Physics, IARI are graphically presented in Figure 3.2. The weekly averages were presented in Annexure I.

3.3 Basic properties of experimental soil

Soils of the experimental block represent a typical alluvium profile of *Yamuna* origin. The entire farm is covered under several soil series. The soil type ranges from sandy loam to clay loam. The texture up to depth of about 150 cm appears almost uniform. As per USDA textural classification, major portion of the area belongs to sandy loam class. Porosity in general is about 40% and soil belongs to good class as far as its permeability is concerned.

3.4 Physical and chemical properties of experimental soil

Soil samples were collected from different layers from surface till the depth of 60 cm along the tree circumference at 120° and analysed to determine physical and chemical properties. Values of the physico-chemical properties are presented in (Table 3.1) and chemical properties like nitrogen, phosphorus, potassium and micro-nutrients such as iron, copper, manganese and zinc are presented in Table 3.2.

Table 3.1 Physico-chemical properties of soil in the experimental field.

Depth (cm)	pH	EC (dS m^{-1})	Textural class
0-30	8.2	0.19	Sandy loam
30-60	7.8	0.15	Sandy loam

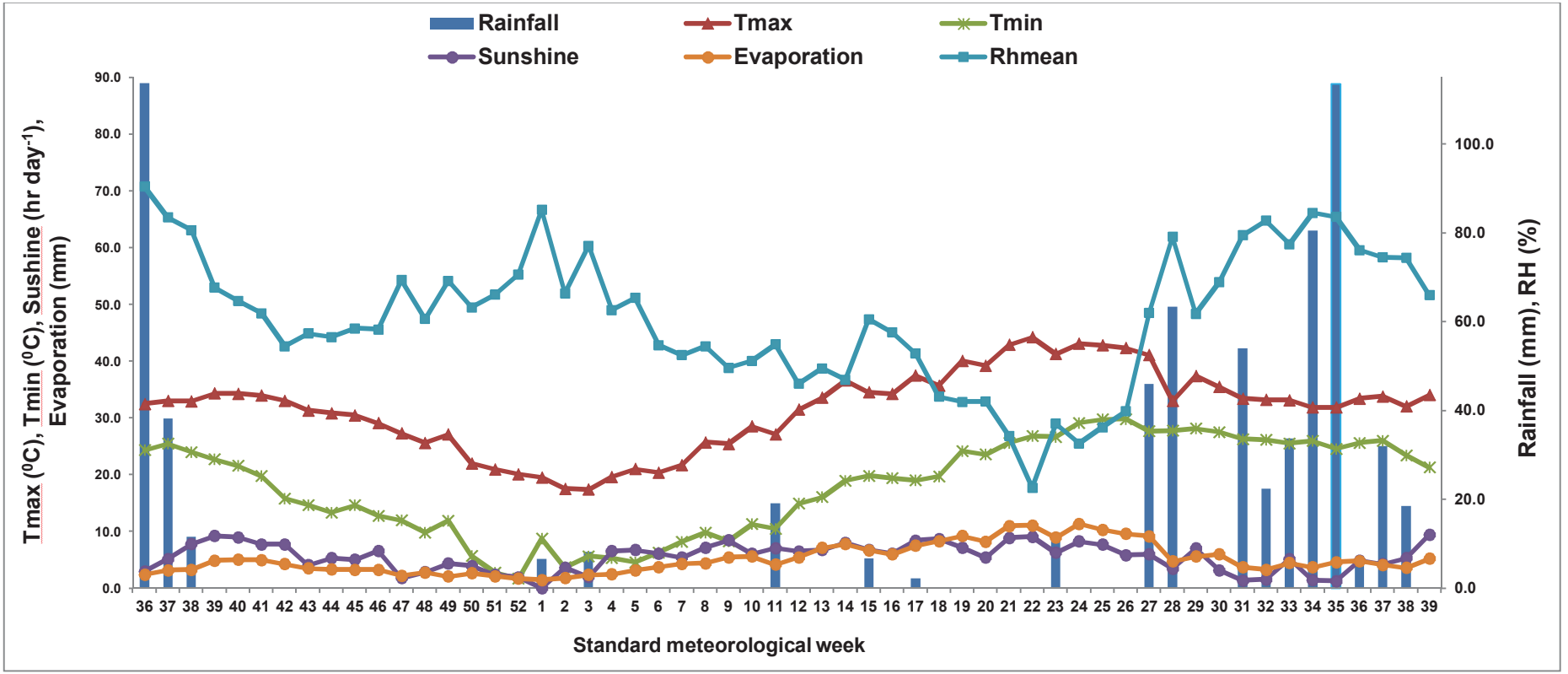

Figure 3.2 Mean weekly weather parameters during September to September (2011-12).

Table 3.2 Chemical properties of soil in the experimental field.

Radial distance (cm)	Depth (cm)	Available nutrient N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})	Fe (ppm)	Cu (ppm)	Mn (ppm)	Zn (ppm)
30	0-30	130.70	30.47	280.03	3.45	2.46	35.38	3.23
	30-60	90.46	25.13	245.32	3.06	2.94	26.29	2.96
60	0-30	109.03	29.96	263.62	3.21	2.32	37.26	3.08
	30-60	84.73	23.24	236.69	2.72	2.72	21.74	2.55

3.5 Experimental plan, material and design

Nitrogen sources (Four): Ammonium sulphate (as ammonical form), calcium nitrate (as nitrate form), mixture of ammonium sulphate and calcium nitrate (as nitrate and ammonical form) and urea. Recommended fertilizers dose was applied in three splits, *i.e*., in September, February and June.

Nitrification inhibitors (Two): Dicyandiamide (DCD) (5% of N-fertilizers) and meliacin (0.1% of N-fertilizers). They were mixed with different nitrogenous fertilizers before application and then applied in the field by ring method. In control treatment no fertilizer and nitrification inhibitor applied. So, there were total 13 treatment combinations

Total treatment combinations:

Notation	Treatment details
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*
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
T_{13}	Urea + *meliacins*

Replications : 3

Plant material : Two-year-old Kinnow budded on *Jatti khatti* plants.

Experimental unit : Two plants per treatment

Fertilizer dose : 1st split in Sept. : 75 g N : 37.5g P : 52.5g K plant^{-1} year^{-1}

2nd split in Feb. : 150g N : 75g P : 105g K plant^{-1} year^{-1}

3rd split in June : 75g N : 37.5g P : 52.5g K plant^{-1} year^{-1}

Experimental Design: Randomized block design.

The three objectives were addressed by conducting following three experiments;

3.5.1 Experiment I: Effect of different nitrogen sources and nitrification inhibitors on soil nitrogen distribution in Kinnow orchard.

Experimental plan, material and design for this experiment was same as mentioned in the paragraph 3.5. Ammonical and nitrate nitrogen distribution was analyzed from the soil samples drawn both lateral (at below dripper and 30 cm away from dripper) and vertical (at 0-30 and 30-60 cm depths) at 30 days after each fertilizer application (*i.e.* in Oct., March and July). A detail description of the procedures adopted for these parameters is presented in the following paragraphs.

For the estimation of mineral N (NH_4^+ and NO_3-$^-$N), portions of ten grams of processed soil samples were extracted with 100 ml of 2 M KCl for 1 h. Extracts were then analyzed for NH_4^+-N by steam distillation with MgO in a micro-Kjeldahl system, and for NO_3^--N after reduction with Devarda's alloy followed by distillation (Bremner and Keeney, 1966)

3.5.2 Experiment II: Influence of nitrogen sources and nitrification inhibitors on vegetative growth, plant physiological processes and biochemical constituents in Kinnow.

Experimental plan, material and design for this experiment was same as mentioned in the paragraph 3.5. The procedures adopted for vegetative growth, plant physiological processes and biochemical constituents is presented in the following paragraphs.

3.5.2.1 Vegetative growth measurements: Vegetative growth parameters measured before and at the end of experiment were as follow:

3.5.2.1.1 Tree height and spread (%): At the initial and at the end of experiment, tree height and spread (N-S; E-W) were measured with the help of pre-marked bamboo pole of 15 m length and measuring tape. Increase in height and spread were calculated by the following formula;

$$\text{Percent increase in length} = \frac{\text{Final length - Initial length}}{\text{Initial length}} \times 100$$

3.5.2.1.2 Shoot growth rate (%): Three uniform shoots per plants were randomly selected and tagged in each treatment. The initial lengths of tagged shoots were measured with the help of meter scale. Later, shoots length was measured at the end of experiment. The per cent increase in shoot length was calculated on the basis of initial and final shoot length.

3.5.2.1.3 Specific leaf area (cm^2 g^{-1} DW): The ten leaf samples from each treated plants were collected and transported in plastic bags to the laboratory. Leaf area was measured by passing sampled leaf, one by one directly in leaf area index meter and recorded the reading for each leaf. The average leaf area was expressed in square centimeters. The samples were then dried and weighed. Specific leaf area was calculated by dividing leaf area with dry weight.

3.5.2.2 Physiological parameters: Following parameters were observed at 45 days after treatment (fertilizer) application.

3.5.2.2.1 Photosynthetic parameters: Photosynthetic and respiration rates and stomatal conductance were measured by using Infra-Red Gas Analyser PS System II (Li-Cor 6200) from 10:00 am to 12:00 noon. The youngest fully emerged leaf of each replication of each treatment was enclosed in a chamber, sealed to avoid gas exchange with the atmosphere and the chamber is placed in sunlight to measure photosynthetic rate (expressed in μmol m^{-2} s^{-1}) respiration rate (expressed in mmol m^{-2} s^{-1}) and stomatal conductance (expressed in mmol m^{-2} s^{-1}).

3.5.2.2.2 Leaf chlorophyll content: The leaf chlorophyll content (chlorophyll a, b and total chlorophyll) was estimated following the method suggested by Hiscox and

Israelstam (1979). Fully matured open leaves were taken as the experimental material. Accurately weighed 100 mg of clean leaf sample was immersed in 10 ml of dimethyl sulphoxide (DMSO) (AR-grade of SRL Chem. Co. Mumbai). The samples were incubated at 70^{o}C for 4 h in hot air oven. Then, it was taken out and 1 ml of the solution was diluted to 5 ml with pure DMSO and the sample was read on a UV-VIS double beam spectrophotometer at 645 and 663 nm wavelengths. Pure DMSO was used as blank. Chlorophyll a, chlorophyll b and total chlorophyll were calculated on fresh weight basis as per the following formulae;

$$\text{Chlorophyll 'a' (mg g}^{-1}\text{f.w.)} = \frac{(12.7 \times OD_{663}) - (2.69 \times OD_{645}) \times \text{volume} \times \text{dilution}}{1000 \times \text{weight of the sample}}$$

$$\text{Chlorophyll 'b' (mg g}^{-1}\text{f.w.)} = \frac{(22.9 \times OD_{645}) - (4.68 \times OD_{663}) \times \text{volume} \times \text{dilution}}{1000 \times \text{weight of the sample}}$$

$$\text{Total chlorophyll (mg g}^{-1}\text{f.w.)} = \frac{(20.7 \times OD_{645}) + (8.02 \times OD_{663}) \times \text{volume} \times \text{dilution}}{1000 \times \text{weight of the sample}}$$

3.5.2.3 Biochemical measurements: Leaves were sampled from Kinnow trees for biochemical analyses at 45 days after treatment application.

3.5.2.3.1 Total soluble sugars: Total sugars in leaf of each treatment were estimated at 45 days after each fertilizer application by the method proposed by Hedge and Hofreiter (1962). The fully mature new leaves samples were collected and washed with double distilled water. Then they were wiped with blotting paper. The leaves of each treatment were excised into pieces and 100 mg of respective samples were weighed in an electronic balance. Then each sample was kept separately in test tube and 5 ml of 2.5 N HCl was put on each test tube. All the test tubes were maintained on a boiling water bath (100°C) for one hour. Then after cooling, the supernatant solution was filtered through Whatman No. 1 filter paper in 100 ml volumetric flask. The residue was re-extracted by boiling with 5 ml of 2.5 N HCL in the water bath at 100°C for one hour and filtering the supernatants through Whatman No. 1 filter paper after cooling. Then after cooling, the supernatants were collected in the volumetric flasks and volume was made up to 100 ml by adding double distilled water. From that supernatant solution, 1 ml of sugar sample was taken in a test tube and freshly prepared 4 ml of anthrone reagent (200 mg of anthrone dissolved in 100

ml of ice cold 95% H_2SO_4) was added. The mixture was heated in a boiling water bath for exactly 8 min. followed by cooling. Optical density of green to dark colour was read at 630 nm. The amount of total sugars in the leaf sample was determined by comparing with the standard curve prepared by taking known concentration of D-glucose in the range of 20-100 µg ml^{-1} and calculated on fresh weight basis according to the following formula.

$$\text{Total leaf sugars (\%)} = \frac{\text{mg of glucose}}{\text{volume of test sample}} \text{ X } 100$$

Preparation of standard curve

Hundred mg of D-glucose was dissolved in 100 ml of double-distilled water. Working standard was prepared by dissolving 10 ml of above stock solution to 100 ml with double-distilled water. From the above solution, 0, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of standards corresponding to 20, 40, 60, 80 and 100 µg of D-glucose were taken in test tubes. Volumes were made up to 1.0 ml followed by addition of freshly prepared 4.0 ml of anthrone reagent. The mixture was then heated in boiling water bath for exactly 8 min. After cooling, absorbance was read at 630 nm in spectrophotometer to prepare standard curve on graph paper sheet by taking absorbance on y-axis and concentration of glucose (µg ml^{-1}) on x-axis.

3.5.2.3.2 Soluble proteins: Soluble protein content of the sample was estimated according to method suggested by Lowry *et al*. (1951). The assay is based on the biuret reaction of protein with alkaline cupric tartarate, forming Cu^{2+}- protein complex. Protein was extracted from leaves sample by cutting into small pieces. One gram of representative samples were weighed and homogenized in chilled mortar and pestle with 5 ml of phosphate buffer (0.1 M; pH 7.5). The mortar and pestle was placed above ice to maintain the temperature of 4ºC during extraction of protein. The homogenate was centrifuged at 10,000 x g for 15 min. at 4ºC. Supernatant was decanted and the residue was discarded. Equal amount of 15% TCA was added to the supernatant and kept overnight at room temperature. Next day, the solution was centrifuged at 3,000 x g for 10 min. at 4ºC and the supernatant was discarded. The precipitated protein was collected by dissolving the residue in 0.1 N NaOH and final volume was made to 10 ml and kept in refrigerator (4^0C) to be used as protein

extract. To prepare the reaction mixture, 0.1 ml of sample extract was taken in test tube and volume was made to 1 ml with double-distilled water.

A tube containing 1 ml of double-distilled water served as a blank. To this, 5 ml of solution C [alkaline cupric tartarate prepared just prior to use by mixing 50 ml of solution A (2% sodium carbonate in 0.1 N NaOH solution) and 1 ml of solution B (mixture of equal volume of 1% $CuSO_4$ and 2% sodium tartarate solution)] were added. The tubes were shaken well by vortexing. After 10 min. 0.5 ml of solution D (1 N Folin-Ciocalteau reagent) was added and mixed well by vortexing till the blue colour developed. Thereafter, the tubes were kept at room temperature for 30 min. and the absorbance was measured at 610 nm wavelength on UV-VIS double beam PC 8 scanning Auto cell spectrophotometer,UVD 3200 (Labomed, INC, USA). The soluble protein content of the sample was quantified by using the standard curve and the result was expressed as mg protein g^{-1} fresh weight of leaves.

Preparation of standard curve for protein estimation

For preparation of standard curve of protein, standard stock solution of Bovine serum albumin (BSA) was prepared by dissolving 6.25 mg of analytical grade BSA in 25 ml double-distilled water. Working solutions were prepared in test tubes by serially diluting the standard solution with double-distilled water to get 25 to 125 µg of BSA per ml. A tube containing 1 ml of double-distilled water served as a blank. To these tubes, 5 ml of solution C [alkaline cupric tartarate prepared just prior to use by mixing 50 ml of solution A (2% sodium carbonate in 0.1 N NaOH solution) and 1 ml of solution B (mixture of equal volume of 1% $CuSO_4$ and 2% sodium tartarate solution)] were added. The tubes were shaken well by vortexing. After 10 min. 0.5 ml of solution D (1 N Folin-Ciocalteau reagent) was added and mixed well by vortexing till the blue colour developed. Then, the tubes were kept at room temperature for 30 min. and the absorbance was measured at 610 nm wavelength on UV-VIS double beam PC 8 scanning Auto cell spectrophotometer, UVD 3200 (Labomed, INC, USA). The readings so obtained were ploted on a graph paper by taking absorbance on y-axis and BSA concentration on x-axis.

3.5.2.3.3 Total free amino acids: Total free amino acids were estimated following the method as described by Lee and Takahashi (1966).

Extraction

One gram of plant material was weighed and grinded in a mortar-pestle with 5 ml of ethanol. The crushed material was centrifuged and the supernatant was carefully transferred. The residue was again ground with 3 ml of ethanol and treated as above. The extraction was done three times to obtain total free amino acids. The final volume was made up to 10 ml with ethanol and was used for estimation of the total free amino acids.

Reagents

1. Citrate buffer: 0.5M (pH 5.6)
2. Ninhydrin: 1% solution in 0.5 M citrate buffer (pH 5.6)
3. Glycerol: 55% solution of glycerol was prepared in distilled water.

Estimation

Following reagents were taken in test tubes in duplicate.

0.2 ml ninhydrin solution.
0.2 ml citrate buffer
1.2 ml glycerol
0.1 ml of sample solution (in control, sample solution was not added)

The volume of the mixture was made up to 5 ml by adding distilled water. Test tubes were then heated in a boiling water bath for 30 min. cooled in running tap water and gently shaken. The absorbance was read using a spectrophotometer at 570 nm. A blank or control was also taken simultaneously for background correction. The quantity of total free amino acids was computed using a standard curve prepared from glycine ($y = 0.0083 x – 0.0358$; $R^2 = 0.9958$) and was expressed as mg/g fresh weight.

3.5.2.3.4 Estimation of nitrate reductase activity (NR; EC 1.6.6.1): Estimation of *in vivo* nitrate reductase activity was done by estimating nitrite formed by the enzyme present in cells and nitrite formed was then diazotized using sulphanilamide in acidic medium and NEDD using the method of Klepper *et al.* (1971). The leaves were cut into 2 mm pieces and after thorough mixing of leaf sample, 0.3 g was weighed and was added to ice cold incubation medium containing 3 ml each of phosphate buffer (0.2 M, pH 7.5) and potassium nitrate solution (0.4 M). To it, 0.2 ml of n-propanol was added. The leaf samples were infiltrated with the solution using a vacuum pump and then incubated in water bath at 33^0C for an hour under dark. At the end of incubation period, tubes were placed in water bath (70-80^0C) for

3-4 min. to stop the enzyme activity and for the complete leaching of the nitrite into the medium. The nitrite was then estimated by taking adequate amount of aliquot in a test tube, to it one ml of sulphanilamide (1% in 1 N HCI) was added. After mixing, 1 ml NEDD (0.02%) was added and again mixed well. Pink colour was formed immediately and after 20 min. the total volume was made up to 4 ml with double distilled water. Absorbance was measured using a double-beam UV visible spectrophotometer (UV57045S) at 540 nm. The calibration curve was prepared using standard sodium nitrite solution (1 O.D. =152.46 µmoles). The enzyme activity was expressed as µmol nitrite formed g^{-1} DW h^{-1}.

3.5.2.3.5 Activity of glutamine synthatase (GS; EC 6.3.1.2): Glutamine synthetase activity was assayed following the method of Mohanty and Fletcher (1980).

Extraction of protein

The fresh fully mature newly leaf samples were collected in ice in the field as mentioned above for NR activity. After cleaning thoroughly the leaf samples were weighed (1 g) and enzyme protein was extracted in the 6 ml ice cold extraction buffer. The extraction step was performed using chilled pestle and mortar in a tray filled with ice. Extract was centrifuged at 10,000 rpm for 20 min in a centrifuge. The supernatant was subsequently used for the assay of enzyme activity.

Assay of enzyme activity

The reaction mixture consisted of (0.1 M Tris 1.25 ml, 10 µM hydroxyl amine 0.6 ml, 100 µM $MgCl_2$ 0.3 ml, , 10 µM ATP 0.2 ml , enzyme extract 50 µl) except sodium glutamate, which is the substrate for the enzyme were added in the assay tubes along with 0.05 ml enzyme extract. All the procedures were done on ice. The tubes were incubated in water bath at 33 ºC for 5 min. to let the solutions attain the desired temperature and then 0.6 ml of sodium glutamate (250 µM) was added to each of the tube. Blank contained all the reagents except sodium glutamate. After 15 min. of incubation reaction was stopped by adding 0.3 ml $FeCl_3$.TCA. The brown precipitate was formed. The volume was made to 6 ml with distilled water and centrifuged at 16,000 rpm for 10 min. in refrigerated centrifuge, to remove the precipitate. The absorbance was recorded at 540 ηm. The GS activity was calculated from the standard curve of γ-glutamyl hydroxymate as the amount of ferric γ-

glutamyl hydroxymate formed (1OD=3.75 µmoles). The GS activity was expressed µmoles γ-glutamyl hydroxymate formed g^{-1} FW h^{-1}.

3.5.2.3.6 Polyphenol oxidase activity: It was assayed by the method of Esterbaner *et al*. (1977). The oxidation of catechol was measured from a reaction mixture containing 2 mL of phosphate buff (pH 6.5), 0.5 mL of enzyme extract, and 1 mL of 0.01 M catechol at 495 nm. Initial absorbance was read at 495 nm and then absorbance was measured at 30 second intervals on a UV-visible spectrophotometer. Enzyme activity was expressed as EU mL^{-1} min^{-1}.

3.5.2.4 Leaf nutrient acquisition: Leaf samples were analysed for macro (N, P and K) and micro nutrients (Fe, Cu, Mn and Zn) at 45 days after each fertilizer application.

Leaf samples for nutrient analysis were collected from plants in each treatment followed by washing of sampled leaves in a series of tap water, 0.1% Teepol® solution, 0.1N HCl and distilled water. In each treatment the sample was packed in labelled paper bags and dried in a hot air oven at temperature of 70ºC till a constant weight was achieved (3 d). Then, the dried sample was grinded with the help of a Willey mill and the ground material was passed through 1 mm mesh sieve. The fine powder as stored in air tight containers and used for digestion of plant sample for tissue nutrient analysis.

3.5.2.4.1 Nitrogen (%): The total nitrogen in plant material was determined by Kjeldahl distillation unit (Jackson, 1967). A dried and finely grinded sample (0.5 g) placed in Kjeldahl tubes added 25 ml concentrated H_2SO_4 was allowed to stand for 30 min. and 5 g of sodium thiosulphate was added and allowed to stand again. First the Kjeldahl's flask was heated slowly till floating continues and it was heated briskly for atleast half an hour after the digest becomes clear. Thereafter cooled water was added along with 40% NaOH and distillation was started. Ammonia was collected in 250 ml conical flask containing boric acid (with mixed indicators). Titration against standard sulphuric acid was carried out after collecting atleast 150 ml of distillate.

Amount (g) of N in the sample = (ml of acid used for sample – ml of acid used for blank) x normality of acid x 14 x 10^{-3})

3.5.2.4.2 Other nutrients: Diacid digestion method was used to determine the extractable phosphorus, potassium, iron, manganese, zinc and copper in plant material. An oven dried and grinded samples was taken (1 g) in each 150 ml conical flask 10 ml of concentrated HNO_3 was added in each flask and allowed to stay overnight for pre-digestion. Three ml of the $HClO_4$ was added next day and the flask was kept on low heat hot plate in a digestion chamber till the contents became colourless. The content was then cooled and approximate 50 ml of distilled water was added and filtrated in 100 ml volumetric flask. The aliquot of the solution were used for determination of P, K, Fe, Zn, Mn and Cu.

Phosphorus: P concentration by Vanado-molybdo-phosphoric yellow colour method (Jackson, 1967). P concentrations of 0, 1, 2, 3, 4, and 5 ppm were prepared by transferring 0, 1, 2, 3, 4, and 5 ml of standard solutions in 50 ml volumetrick flask; added 10 ml of vanadomolybdate solution and the volume was made to the mark with double distilled water. The transmittance of standard P concentrations was read after 30 min. at 420 nm with the help of spectrophotometer. Then the curve was prepared by plotting absorbance against the concentration. The total phosphorus in plant sample was estimated by the use of vanadomolybdate solution. 5 ml of extract obtained through diacid method was taken in 50 ml volumetrick flask and the procedure used for standards was followed. The P concentration was calculated by using standard curve.

P in % = (Concentration of P from curve in (ppm) x dilution factor) / 10000)

Potassium: Potassium was determined with the help of flame photometer (Jackson, 1967). The standard curve was prepared by setting instrument at highest concentration using standard filter. The extract obtained by diacid method was diluted to the suitable concentration range and finally the samples were read in flame photometer using filter for K.

K in % = (Concentration of K from curve in ppm x dilution factor) / 10000

Micro-nutrients (Zn, Fe, Cu and Mn): The same extract obtained by diacid method was used to determine Fe, Mn, Zn and Cu in plant material by using atomic absorption spectrophotometer (AAS) GBC Avanta. Standard concentration of each element was prepared as:

Working standard solutions of Zn were prepared by taking 0, 0.5, 1, 2 and 2.5 ml of stock solution (10 ppm Zn) to a series of 100 ml volumetric flask and diluted each to the mark with double distilled water to have concentration of Zn in solution of 0, 0.1, 0.2, 0.4, and 0.5 ppm. The standard working solutions were fed into AAS and the standard curve was prepared by plotting AAS readings against Zn concentrations. The standard curves were prepared by plotting AAS readings against each element concentration. The other standards (Fe, Cu, Mn) followed the same preparation as prescribed above for Zn. The concentrations of zinc, copper, iron and manganese in plant material were read from AAS were subject to following calculation to get the values.

Micronutrients (ppm) = Conc. of micronutrients from curve (ppm) x dilution factor

3.5.3 Experiment III: Determination of soil biological activities with respect to nitrogen sources and nitrification inhibitors in rhizosphere under Kinnow plants.

Experimental plan, material and design for this experiment were same as mentioned in the paragraph 3.5. The procedures adopted for determination of soil biological activities is presented in the following paragraphs. Observations on soil biological activities were measured during the month of October in 2012 (*i.e.* at the end of experiment). Soil samples from surface depth (0-15) were taken in small polythene bags from each plot by core sampler. The soil samples were ground and passed through 2 mm mesh-sieve, and analysed for microbial parameters, *viz.*, alkaline phosphatase activity, dehydrogenase activity, β-glucosidase activity, Fluorescein diacetate Assay, Microbial biomass carbon and Urease activity. Dehydrogenase was estimated as described by Casida *et al.* (1964). Alkaline phosphatases was analyzed following the method of Tabatabai and Bremner (1969). Urease activity was assessed as the rate of urea hydrolysis in the soil samples by determining the urea remaining (unhydrolysed) following the method modified by Douglas and Bremner (1970), fluorescein diacetate (FDA) hydrolysis (Green *et al*., 2006), β-Glucosidase (Eivazi and Tabatabai, 1988) and microbial biomass C (Nunan *et al*., 1998) were measured at the initial and end of experimentation.

3.6 Installation of drip system

An online drip irrigation system was installed for Kinnow orchard. The control head of the system consisted of sand filter, flow control valve, screen filter,

pressure gauges etc. A PVC sub-mainline (50 mm outer diameter, 4 kg/cm^2 working pressure) already was installed in the experimental field area. Lateral lines of LDPE (16 mm diameter) were connected to the sub main line for the irrigation of Kinnow plant. The lateral lines were placed along the Kinnow row having four online emitters of four litres per hour (4 l/h) capacity surrounding the tree. Each lateral line was provided with flow control valve at the start of the line to achieve specific irrigation operation.

3.7 Irrigation scheduling

Irrigation was scheduled daily as per consumptive water requirement calculated as per formula given below. There was a good correlation between plant water requirements and evaporation. Citrus trees use approximately 70 per cent of evaporation. Water use is proportional to the area of exposed leaf, which relates to the area of land covered by the canopy. The irrigation was given based on the formula given below:

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

3.8 Statistical analysis

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. The results are presented by tables and graphics.

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4. Results

Results of the field experiment entitled "Effect of nitrogen sources and nitrification inhibitors on nitrogen dynamics and plant performance of Kinnow mandarin" conducted during winter, autumn and summer seasons of 2011-12 at the Indian Agricultural Research Institute, New Delhi are being presented and described in this chapter. The data related to various criteria used for treatment evaluation were analysed statistically using standard statistical methods to test their significance. The results for all the treatments are described in this chapter. Wherever necessary, the data recorded have also been presented graphically to provide better understanding of important trends. The experimental findings of the present investigation are being presented under the appropriate sub-heads.

4.1 Ammonical and nitrate nitrogen distribution

The amounts of NH_4^+–N and NO_3^-–N in the soil were measured in order to estimate the residual concentration of these anions in the upper and deeper soil layers.

4.1.1 NH_4^+ nitrogen distribution in soil at 0-30 cm depth

The concentration of NH_4^+-N in soil at 0-30 cm depth below and 30 cm away from drippers was significantly higher when respective N-fertilizers treated with nitrification inhibitors (both DCD and meliacins) than they were applied individually except nitrate nitrogen fertilizers in all split applications. The NH_4^+ concentrations were significantly lower in control and nitrate N-fertilizers treatments, whereas, significantly higher in the AS + DCD treatment (44.1, 55.7, 42.7 mg kg^{-1} soil below drippers and 37.8, 41.9, 36.0 mg kg^{-1} soil at 30 cm away from drippers in all three split applications respectively) followed by urea + DCD and AS + meliacins treatments. Among N-fertilizers alone, the ammonical N-fertilizer form retained more NH_4^+-N in the upper soil profile (0-30 cm) than the other form of N-fertilizers used in this study (Table 4.1).

4.1.2 NH_4^+ nitrogen distribution in soil at 30-60 cm depth

The concentration of NH_4^+ in soil at 30-60cm depth below and 30cm away from drippers followed a similar trend as of 0-30 cm depth but the concentration of the NH_4^+

Table 4.1 Distribution of NH_4^+-N from different nitrogen fertilizers with or without nitrification inhibitors in the Kinnow soil profile.

Treatment	First split application				Second split application				Third split application			
	Below dripper		30 cm away from dripper		Below dripper		30 cm away from dripper		Below dripper		30 cm away from dripper	
	0-30[#]	30-60	0-30	30-60	0-30	30-60	0-30	30-60	0-30	30-60	0-30	30-60
T1	12.3±0.7^{f}	8.0±0.6^{f}	10.4±0.4^{f}	6.9±0.3^{d}	10.7±0.4^{g}	8.4±0.9^{d}	7.8±0.4^{e}	7.3±0.3^{c}	9.7±0.4^{e}	8.3±0.3^{g}	9.5±0.4^{g}	5.8±0.3^{f}
T2	30.9±0.7^{cd}	21.1±0.6^{bc}	29.3±0.6^{bc}	19.1±0.4^{a}	40.7±0.9^{c}	20.6±2.0^{a}	31.1±0.7^{c}	20.7±1.6^{a}	28.3±0.6^{c}	18.8±0.4^{bcd}	27.7±0.6^{c}	15.2±0.3^{cd}
T3	13.8±0.6^{f}	9.0±0.7^{f}	9.6±0.4^{f}	5.6±0.5^{d}	9.7±0.4^{g}	7.1±0.3^{d}	7.9±0.4^{e}	7.0±0.9^{c}	11.2±0.5^{e}	7.6±0.3^{g}	9.7±0.4^{g}	5.1±0.2^{f}
T4	25.2±0.9^{e}	16.8±1.3^{e}	20.9±0.5^{e}	12.4±0.5^{c}	26.1±0.7^{f}	14.7±0.2^{c}	20.9±0.5^{d}	14.7±1.6^{b}	22.9±0.6^{d}	13.9±0.3^{f}	20.0±0.5^{f}	11.3±0.3^{e}
T5	28.4±1.2^{de}	19.2±0.6^{cde}	25.7±1.1^{d}	19.9±1.3^{a}	34.5±1.5^{de}	18.9±0.5^{ab}	30.7±1.5^{c}	16.4±1.5^{b}	28.9±1.3^{c}	17.5±0.8^{de}	23.5±1.1^{e}	13.9±0.7^{d}
T6	44.1±1.5^{a}	24.2±1.7^{a}	37.8±1.3^{a}	20.0±1.3^{a}	55.7±1.9^{a}	20.2±0.7^{a}	41.9±1.4^{a}	23.5±0.8^{a}	42.7±1.4^{a}	23.2±0.8^{a}	36.0±1.2^{a}	23.6±0.8^{a}
T7	41.0±0.9^{ab}	23.3±1.1^{ab}	35.2±0.8^{a}	19.5±0.5^{a}	50.3±1.2^{b}	21.5±1.5^{a}	36.8±0.8^{b}	21.5±1.0^{a}	37.0±0.9^{b}	20.1±0.5^{b}	34.9±0.8^{a}	19.8±0.5^{b}
T8	13.5±0.6^{f}	8.9±0.5^{f}	10.5±0.3^{f}	5.9±0.7^{d}	9.9±0.2^{g}	7.8±0.3^{d}	7.3±0.2^{e}	6.5±0.2^{c}	11.7±0.3^{e}	9.1±0.2^{g}	11.0±0.3^{g}	5.7±0.5^{f}
T9	12.7±0.7^{f}	9.4±0.7^{f}	9.9±0.4^{f}	6.2±0.4^{d}	10.7±0.4^{g}	8.2±0.3^{d}	6.1±0.3^{e}	7.1±0.3^{c}	10.0±0.4^{e}	8.6±0.3^{g}	9.1±0.4^{g}	5.2±0.4^{f}
T10	31.9±1.8^{c}	20.6±0.8^{bcd}	28.4±1.6^{c}	16.2±0.9^{b}	32.9±0.1^{e}	16.1±0.7^{bc}	31.0±0.1^{c}	15.0±0.6^{b}	28.0±1.4^{c}	18.3±0.1^{cd}	27.2±0.1^{cd}	15.5±0.8^{c}
T11	29.1±1.6^{cd}	18.3±0.7^{de}	25.4±1.0^{d}	14.5±0.8^{bc}	36.8±1.4^{d}	16.9±1.3^{bc}	32.2±1.2^{c}	17.2±1.5^{b}	27.0±1.0^{c}	16.0±0.6^{e}	25.3±0.9^{de}	13.9±0.5^{d}
T12	42.9±1.0^{ab}	23.9±0.6^{a}	36.5±0.9^{a}	20.6±1.0^{a}	52.5±1.2^{ab}	21.7± 1.0^{a}	40.0±0.9^{a}	21.8±1.1^{a}	38.1±0.9^{b}	22.0±0.5^{a}	35.2±0.8^{a}	20.0±0.5^{b}
T13	40.7±1.9^{b}	22.9±1.0^{ab}	31.7±1.6^{b}	21.0±1.1^{a}	42.3±1.9^{c}	20.0±0.9^{a}	33.4±1.5^{c}	23.0±1.0^{a}	35.6±1.6^{b}	19.9±0.9^{bc}	30.3±1.4^{b}	18.4±0.8^{b}
SE(d)	1.67	1.35	1.29	1.08	1.64	1.41	1.33	1.42	1.42	0.77	1.16	0.78
LSD (P ≤ 0.05)	3.45	2.78	2.67	2.22	3.39	2.91	2.75	2.93	2.94	1.59	2.40	1.60

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. [#] Depth in cm

Table 4.2 Distribution of NO_3^--N (mg kg^{-1} soil) from different nitrogen fertilizer with or without nitrification inhibitors in the Kinnow soil profile.

Treatment	First splits application				Second splits application				Third splits application			
	Below dripper		30 cm away from dripper		Below dripper		30cm away from dripper		Below dripper		30cm away from dripper	
	0-30[#]	30-60	0-30	30-60	0-30	30-60	0-30	30-60	0-30	30-60	0-30	30-60
T1	13.9±0.6^{e}	10.1±0.4^{g}	12.7±0.6^{f}	8.1±0.4^{h}	11.1±0.5^{i}	9.9±0.4^{g}	8.1±0.4^{g}	25.3±1.1^{b}	11.1±0.5^{h}	8.1±0.3^{f}	12.4±0.6^{h}	7.4±0.3^{g}
T2	28.3±0.6^{b}	21.51±0.5^{de}	25.9±0.6^{b}	22.0±0.5^{cd}	32.8±0.7^{de}	25.6±0.6^{bcd}	26.2±0.6^{d}	22.1±0.5^{cde}	24.7±0.5^{cd}	20.3±0.4^{de}	24.8±0.5^{bc}	24.6±0.5^{cd}
T3	20.4±0.9^{d}	29.0±1.3^{a}	17.3±0.8^{e}	26.5±1.2^{a}	25.2±1.1^{h}	33.6±1.5^{a}	19.7±0.9^{f}	30.6±1.4^{a}	17. 9±0.8^{g}	29.7±1.3^{a}	16.9±0.8^{g}	28.7±1.3^{a}
T4	23.8±0.6^{c}	25.6±0.6^{bc}	21.1±0.5^{d}	23.8±0.6^{bc}	26.6±0.7^{gh}	27.6±0.7^{b}	23.8±0.6^{de}	24.7±0.6^{bc}	19. 9±0.5^{fg}	22.5±0.6^{cd}	21.0±0.5^{ef}	25.7±0.6^{bc}
T5	24.0±1.1^{c}	25.3±1.1^{bc}	25.1±2.1^{bc}	22.1±1.1^{cd}	30.8±1.4^{ef}	25.7±1.1^{bcd}	25.9±1.2^{d}	22.7±1.1^{cd}	21.7±1.0^{ef}	26.1±1.1^{b}	22.5±1.1^{cde}	25.8±1.2^{bc}
T6	33.9±1.1^{a}	18.8±0.6^{f}	27.3±0.9^{b}	16.8±0.6^{g}	41.0±1.4^{a}	20.5±0.7^{f}	37.0±1.3^{a}	18.3±0.6^{g}	31.4±1.1^{a}	19.1±0.6^{e}	31.1±1.1^{a}	19.9±0.7^{f}
T7	31.9±0.7^{a}	19.4±0.4^{ef}	30.7±0.7^{a}	17.3±0.4^{fg}	38.9±0.9^{ab}	21.0±0.5^{f}	34.7±0.8^{ab}	19.7±0.5^{efg}	28.9±0.7^{b}	18.8±0.4^{e}	29.4±0.7^{a}	20.2±0.5^{f}
T8	23.4±0.6^{c}	27.6±0.7^{ab}	21.4±0.8^{d}	25.2±0.6^{ab}	25.8±0.6^{h}	32.3±0.8^{a}	21.6±0.5^{ef}	28.7±0.7^{a}	19.1±0.5^{g}	31.7±0.8^{a}	19.1±0.5^{fg}	29.4±0.7^{a}
T9	22.6±0.9^{cd}	28.4±1.2^{a}	22.5±1.0^{cd}	26.1±1.2^{a}	26.5±1.1^{gh}	31.5±1.3^{a}	19.3±0.9^{f}	29.2±1.3^{a}	18.0±0.7^{g}	32.1±1.3^{a}	18.5±0.8^{g}	27.9±1.3^{ab}
T10	27.5±0.1^{b}	23.6±0.1^{cd}	22.6±0.1^{cd}	19.1±0.1^{ef}	29.4±0.1^{fg}	24.6±0.1^{cde}	25.5±0.1^{d}	21.5±0.1^{def}	25.9±0.1^{c}	23.1±0.8^{c}	22.5±0.1^{de}	21.9±0.1^{ef}
T11	27.0±1.0^{b}	23.8±0.9^{cd}	21.9±0.8^{d}	20.2±0.8^{de}	29.1±1.1^{fg}	26.0±1.0^{bc}	24.1±0.9^{de}	21.6±0.8^{def}	23.4±0.9^{de}	23.3±0.9^{c}	23.2±0.9^{bcde}	23.1±0.9^{de}
T12	28.7±0.7^{b}	21.6±0.5^{de}	27.3±0.6^{b}	20.1±0.5^{de}	36.6±0.9^{bc}	22.3±0.5^{ef}	32.9±0.8^{b}	19.3±0.5^{fg}	26.1±0.6^{c}	21.2±0.5^{cde}	25.2±0.6^{b}	23.0±0.5^{de}
T13	28.3±1.3^{b}	22.2±1.0^{d}	25.8±1.2^{b}	18.9±0.9^{efg}	34.7±1.6^{cd}	23.1±1.1^{def}	29.1±1.3^{c}	18.1±0.8^{g}	25.7±1.2^{c}	23.7±1.1^{bc}	24.6±1.1^{bcd}	22.8±1.0^{de}
SE(d)	1.23	1.17	1.37	1.07	1.47	1.28	1.26	1.23	1.09	1.25	1.11	1.21
LSD (P≤ 0.05)	2.55	2.41	2.83	2.23	3.04	2.64	2.60	2.54	2.25	2.59	2.30	2.51

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.

[#] Depth in cm

was lower. The NH_4^+ concentrations as depicted in Table 4.1 were significantly lower in control and nitrate N-fertilizer treatments whereas, significantly higher in the AS + DCD treatment (24.2, 20.2, 23.2 mg kg^{-1} soil below drippers and 20.0, 23.5, 23.6 mg kg^{-1} soil at 30 cm away from drippers in all three split applications, respectively) followed by urea + DCD and AS + meliacins treatment (Table 4.2).

4.1.3 NO_3^- nitrogen distribution nitrification inhibitors (*i.e*. DCD and meliacins) slow down nitrification and the NO_3^--N slowly available for longer periods which lower the chances of leaching.

The concentration of NO_3^--N in soil at 0-30 cm depth below and 30 cm away from drippers was significantly higher in the treatments containing nitrification inhibitors, *i.e.,* DCD and meliacins except nitrate nitrogen fertilizers in all split applications. This implies that nitrification leads to formation of NO_3^- within a few days when fertilizers not treated with nitrification inhibitors. This NO_3^--N utilized by the plants and excess NO_3^- leached to the ground level. Whereas, when N-fertilizers treated with nitrification inhibitors (*i.e*. DCD and meliacins) nitrification slow down and the NO_3^--N slowly available for longer periods which lower the chances of leaching. The NO_3^--N concentrations as presented in Table 4.2 were significantly lower in control whereas, significantly higher in the AS + DCD treatment (33.9, 41.0, 31.4 mg NO_3^--N kg^{-1} soil below drippers and 27.3, 37.0, 31.1 mg NO_3^--N kg^{-1} soil at 30 cm away from drippers in all three split applications respectively) followed by urea + DCD and AS + meliacins treatment. Among N-fertilizers alone, the ammonical N-fertilizer form retained more NO_3^--N in the upper soil profile (0-30 cm) than the other form of N-fertilizers at 30 days after application.

4.1.4 NO_3^- nitrogen distribution in soil at 30-60 cm depth

The concentration of NO_3^--N in soil at 30-60 cm depth below and 30 cm away from drippers was significantly higher in all treatments not containing nitrification inhibitors, *i.e.,* DCD and meliacins in all split applications. The concentration of NO_3^- N in soil at 30-60 cm depth found lower in treatments containing nitrification inhibitors, as most of the NO_3^- N remain in the upper soil layer (0-30 cm depth) due to slow nitrification. Between different treatments the lowest NO_3^--N found in AS + DCD (18.8, 20.5, 19.1 mg NO_3^--N kg^{-1} soil below drippers and 16.8, 18.3, 19.9 mg

NO_3^--N kg^{-1} soil at 30 cm away from drippers in all three split applications respectively) followed by AS + meliacins and AS + DCD treatment.

The highest NO_3^--N found in T_3 and T_9 treatments at below and 30 cm away from drippers during the first split application whereas, in second and third split application highest NO_3^--N found in T_3, T_8 and T_9 treatments at below and 30 cm away from drippers.

4.2 Vegetative growth measurements

Data pertaining to growth attributes, *viz*., per cent increase in tree height, spread, shoot length and specific leaf area as influenced by different nitrogen sources and nitrification inhibitors are presented in the Fig. 4.1, Table 4.3 and Appendix II. At the end of experiment, significant differences in vegetative growth of Kinnow was found among treatments. DCD and meliacins treated AS and urea resulted in higher plant's growth (per cent increase in tree height, spread, shoot lengths and specific leaf area) then their individual application.

When different forms of N-fertilizers were amended with DCD and meliacins, the percentage increase in tree height found significantly higher in treatment AS + DCD (44.05%) which was at par with AS + meliacins, urea + meliacins and urea + DCD. Tree spread (E-W) found statistically highest in treatment T_7 *i.e.*, 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}) (Fig. 4.1). A perusal of data presented in Table 4.3 revealed that as far as specific leaf area is concerned it was found statistically higher in treatment T_7 (123.86 cm^2 g^{-1}) followed by T_6 and T_{13}. The maximum value of shoot growth rate (247.39%) was observed under 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).

When different N-forms alone (without NI) were compared than all the growth parameter found higher in treatment receiving urea and ammonium sulphate.

4.3 Physiological parameters: It was performed at 45 days after each fertilizer application.

Table 4.3 Effect of different nitrogen sources with or without nitrification inhibitors on shoot growth rate (%) and specific leaf area (cm^2 g^{-1}) in Kinnow mandarin.

Treatment	Shoot growth rate (%)	Specific leaf area (cm^2 g^{-1})
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

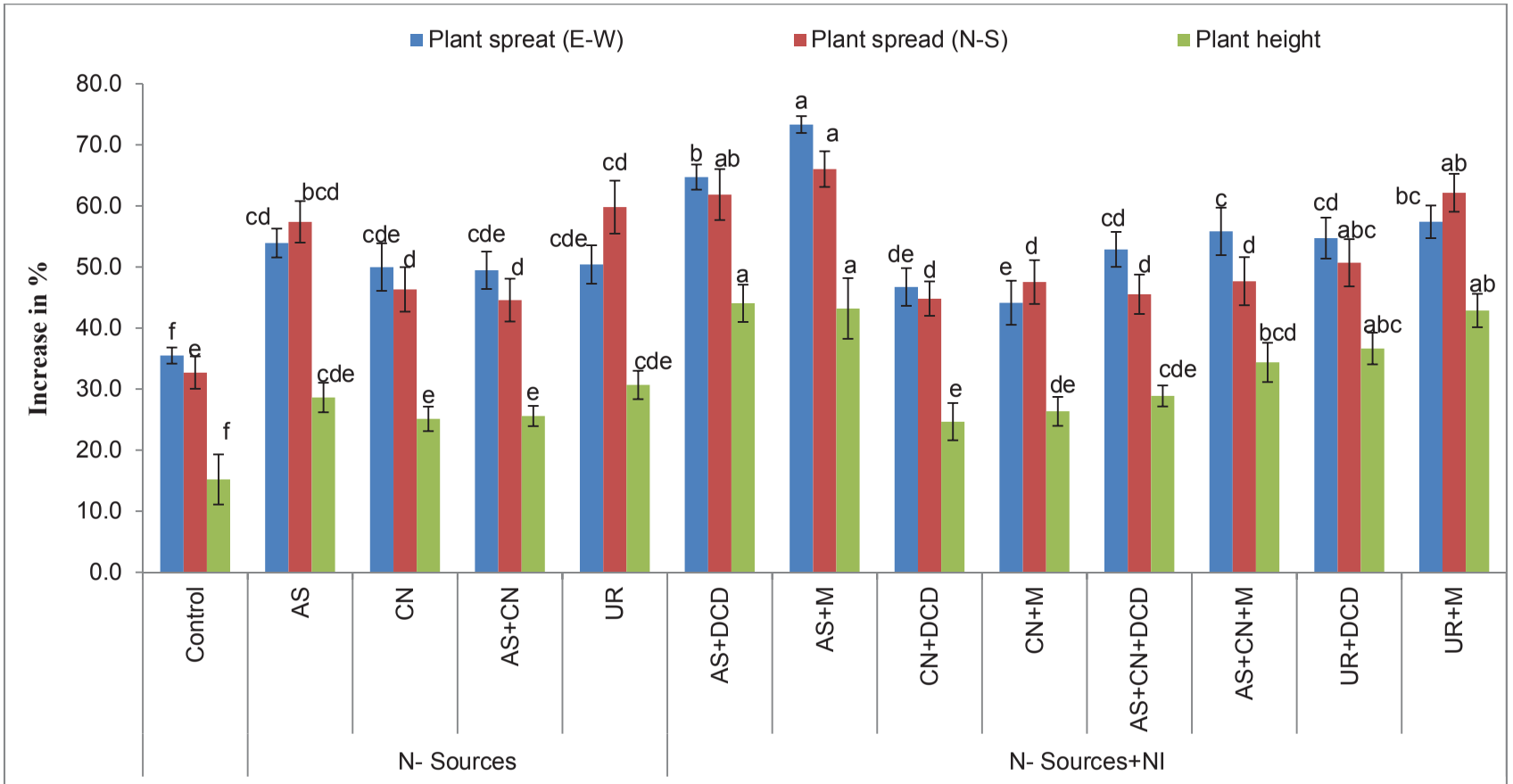

Fig. 4.1 The percentage increase in plant spread (E-W & N-W) and plant height due to different nitrogen sources with or without nitrification inhibitors in Kinnow mandarin. Data represent the mean ± standard error of three independent determinates. Means within a column that did not differ significantly at 5% level of significance when compared with Fisher's Least Significant Difference are followed by the same superscript letters.

4.3.1 Leaf chlorophyll content

It is evident from data (Fig. 4.2 and Table 4.4) that different N-sources and nitrification inhibitors exerted significant variation in a, b and total chlorophyll content in leaves of Kinnow during all three split application of fertilizers.

In the current study, a, b and total chlorophyll content were higher with application of AS and urea among the untreated N-fertilizers but, addition of nitrification inhibitors (DCD and meliacins) outperformed these alone N-fertlilizers treatment. 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 mg g^{-1} Fw chl a, chl b and total chlorophyll content during first, second and third split application, respectively).

4.3.2 Photosynthetic rate

Kinnow mandarin exhibited significant response when treated with different nitrogen sources (fertilizers) and nitrification inhibitors for photosynthetic rate at all three season, as compared to control treatment (Table 4.5). Nitrification inhibitors had positive response to photosynthetic rate at all three split fertilizer application. When different N-fertilizers were 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^{-1} with AS + DCD during first, second and third split application, respectively), however, it remained statistically similar to AS + meliacins, UR + meliacins and UR + DCD. Among the untreated N-fertilizers, AS followed by urea showed higher photosynthetic rate. The control exhibited the lowest photosynthetic rate.

4.3.3 Transpiration rate

The observations recorded for leaf transpiration rate of Kinnow mandarin, as influenced by different nitrogen sources and nitrification inhibitors, revealed that untreated N-fertilizers had comparatively lesser leaf respiration rate than respective N-fertilizers treated with NI (DCD and meliacins) in all three split fertilizer application and lowest in control (Table 4.5).

Application of AS + meliacins registered significantly maximum values of transpiration rate, *i.e.*, 2.15 and 2.13 mmol m^{-2} s^{-1} during winter and summer split

Table 4.4. Effect of different nitrogen sources with or without nitrification inhibitors on chlorophyll a and chlorophyll b (mg g^{-1} Fw) content in Kinnow.

Treatment	Chlorophyll a (mg g^{-1} Fw)			Chlorophyll b (mg g^{-1} Fw)		
	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}
T8 (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}
T9 (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}
T10 (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}
T11 (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}
T12 (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}
T13 (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.

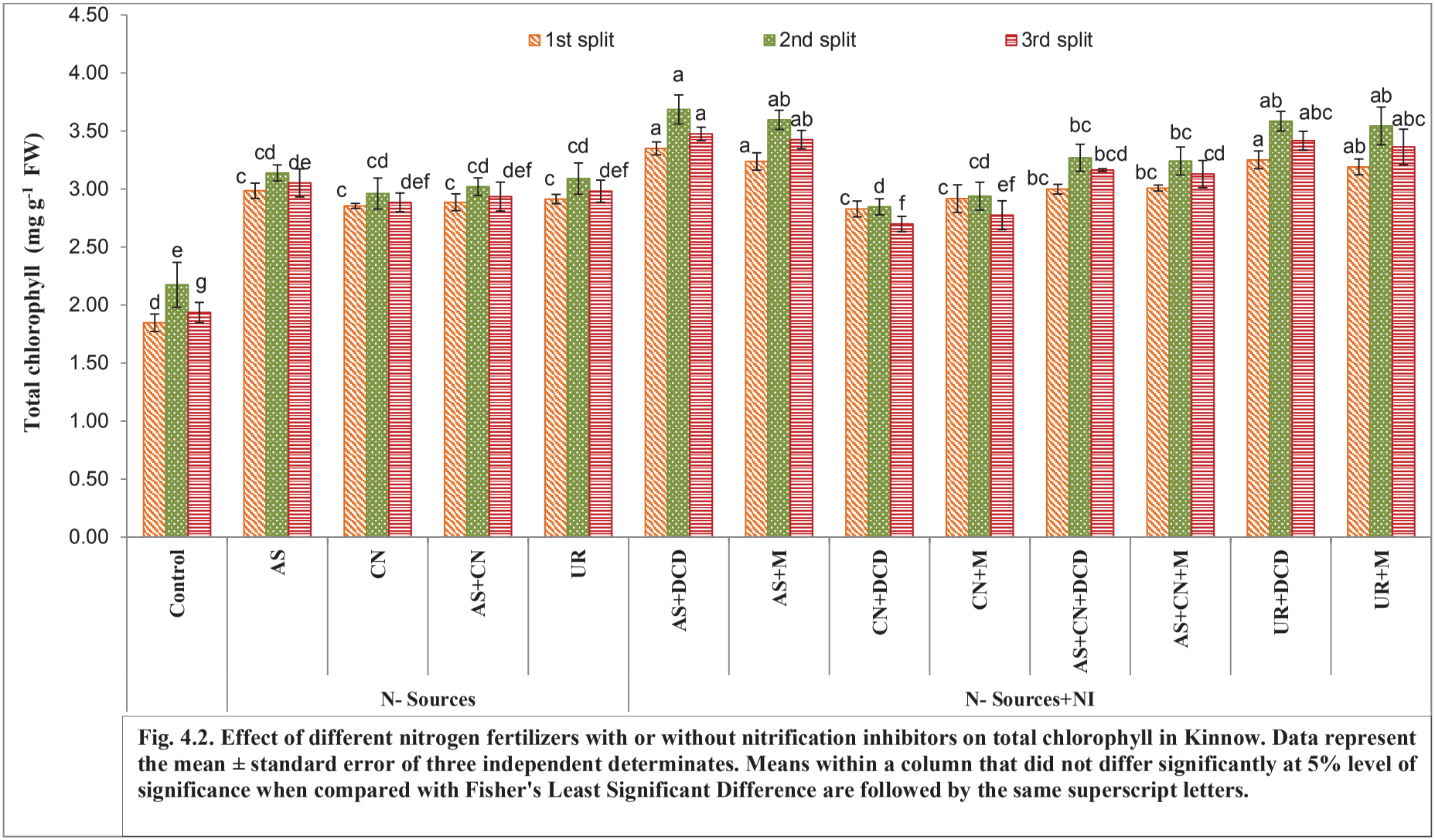

Fig. 4.2. Effect of different nitrogen fertilizers with or without nitrification inhibitors on total chlorophyll in Kinnow. Data represent the mean ± standard error of three independent determinates. Means within a column that did not differ significantly at 5% level of significance when compared with Fisher's Least Significant Difference are followed by the same superscript letters.

Table 4.5 Physiological variables of Kinnow mandarin influenced by different nitrogen sources with or without nitrification inhibitors.

Treatment	Photosynthesis rate (µmol m^{-2} s^{-1})			Transpiration rate (mmol m^{-2} s^{-1})			Stomatal conductance (mmol m^{-2} s^{-1})		
	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}
T8 (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}
T9 (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}
T10 (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}
T11 (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}
T12 (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}
T13 (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.

application respectively, whereas, in winter (second split) it was found higher under AS + DCD treatment (2.35 mmol m^{-2} s^{-1}). 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.

4.3.4 Stomatal conductance

Data on stomatal conductance is presented in Table 4.5. It showed the same trend as presented in the photosynthetic rate. Like photosynthetic rate similarly, mean values of stomatal conductance at all three season was significantly higher with the treatment AS + DCD (37.52, 38.41 and 36.82 mmol m^{-2} s^{-1}) during first, second and third split application respectively, however, it remained statistically similar to AS + meliacins, UR + meliacins and UR + DCD. Among the untreated N-fertilizers, AS followed by urea showed higher stomatal conductance. The control exhibited the lowest stomatal conductance.

4.4 Biochemical measurements: Leaf samples from Kinnow trees were analysised at 45 days after each fertilizer application for biochemical measurments. The values of all biochemical parameters also differed significantly with various N-sources and nitrification inhibitors (Fig. 4.3-4.4, Table 4.6).

4.4.1 Total soluble sugars, proteins and free amino acids in leaves

All the N-sources with and without nitrification inhibitors produced significantly higher total soluble sugars, proteins and free amino acids in leaves of Kinnow over control (Fig. 4.3-4.4). 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^{-1} leaf dry wt) during winter, autumn and summer, respectively followed by ammonium sulphate treated with meliacins.

4.4.2 Total free amino acids

A perusal of data presented in Table 4.6 revealed that the total free amino acid influenced significantly under different N-sources and nitrification inhibitors. Significantly higher free amino acids (2.53, 2.67 and 2.51 µmol g^{-1}) were found in AS + DCD treatment during winter, autumn and summer, respectively.

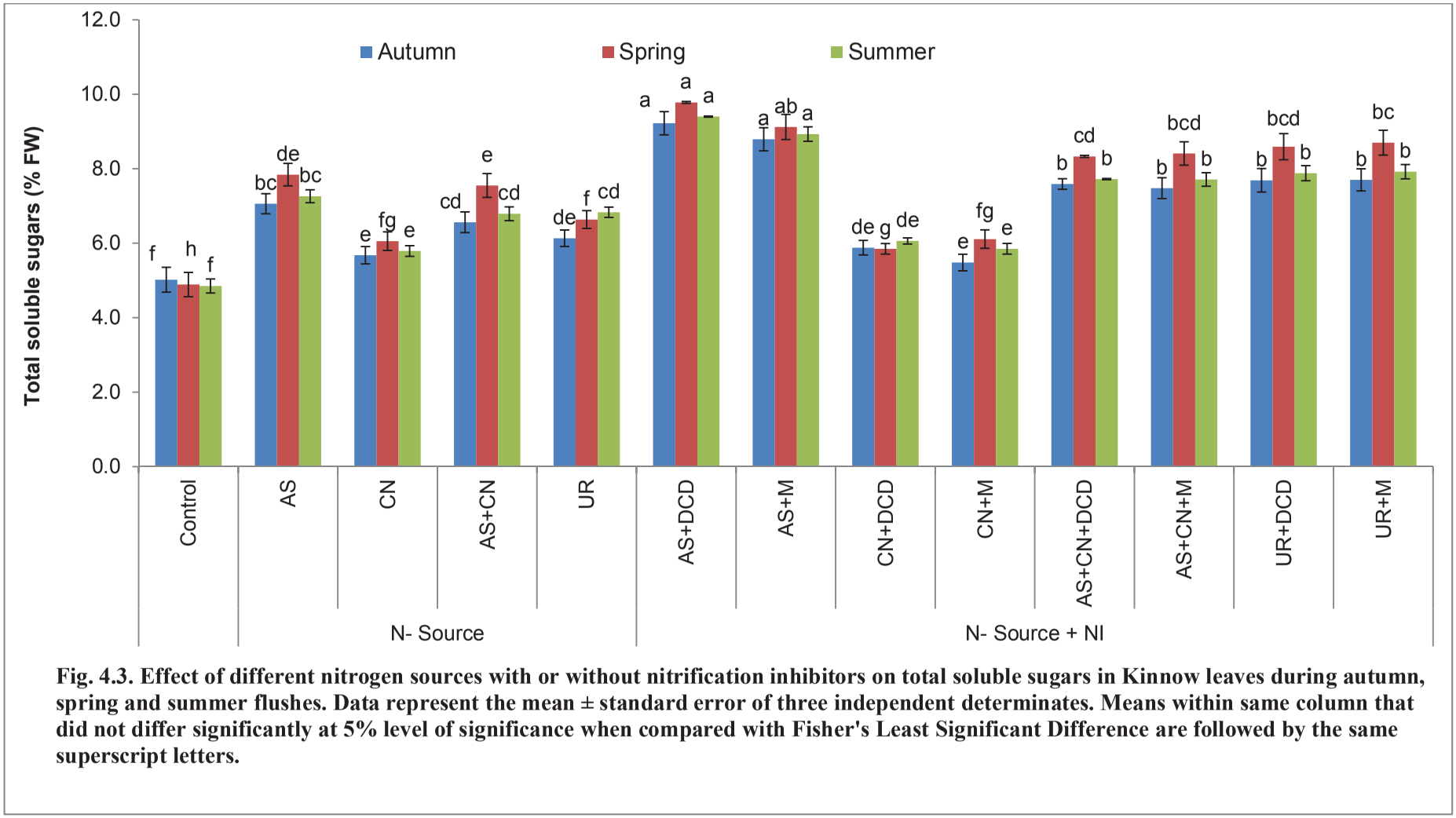

Fig. 4.3. Effect of different nitrogen sources with or without nitrification inhibitors on total soluble sugars in Kinnow leaves during autumn, spring and summer flushes. Data represent the mean ± standard error of three independent determinates. Means within same column that did not differ significantly at 5% level of significance when compared with Fisher's Least Significant Difference are followed by the same superscript letters.

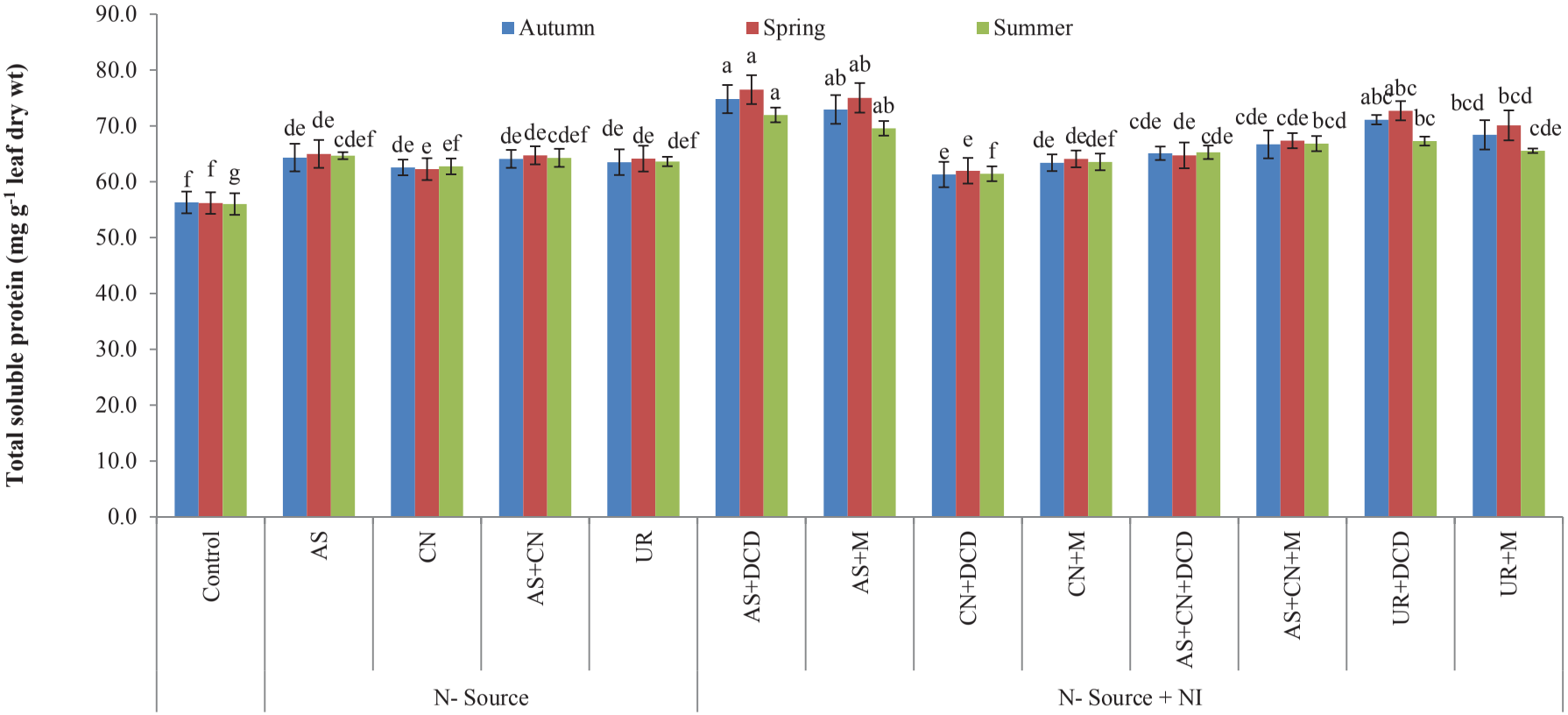

Fig. 4.4. Effect of different nitrogen sources with or without nitrification inhibitors on total soluble protein in Kinnow leaves during autumn, spring and summer flushes. Data represent the mean ± standard error of three independent determinates. Means within same column that did not differ significantly at 5% level of significance when compared with Fisher's Least Significant Difference are followed by the same superscript letters.

Table 4.6 Effect of different nitrogen sources with or without nitrification inhibitors on nitrate reductase (NR) (µmol nitrite formed g^{-1} FW h^{-1}) activity and free amino acids (µmol g^{-1}) content in leaves of Kinnow trees.

Treatment	Nitrate reductase (NR) activity			Free amino acids		
	Autumn	Spring	Summer	Autumn	Spring	Summer
T_1 (Control)	212.00±7.29^{f}	206.52±7.10^{g}	200.98±6.91^{d}	1.80±0.07^{h}	1.84±0.16^{f}	1.85±0.08^{e}
T_2 (AS)	497.02±19.10^{a}	478.31±18.38^{a}	469.68±18.05^{a}	1.91±0.04^{fgh}	1.98±0.04^{ef}	1.94±0.08^{e}
T_3 (CN)	314.07±7.10^{de}	276.64±12.59^{f}	309.98±17.07^{c}	2.07±0.02^{ef}	2.02±0.09^{def}	1.97±0.06^{de}
T_4 (AS+ CN)	376.58±15.98^{b}	383.60±16.28^{bc}	324.44±13.77^{c}	2.15±0.05^{cde}	1.86±0.05^{f}	1.82±0.08^{e}
T_5 (UR)	353.71±12.75^{bcd}	389.96±14.06^{b}	408.95±14.75^{b}	1.87±0.03^{gh}	1.92±0.08^{f}	1.90±0.06^{e}
T_6 (AS+DCD)	368.95±13.13^{b}	372.65±12.62^{bcd}	375.34±12.71^{b}	2.53±0.04^{a}	2.67±0.09^{a}	2.51±0.04^{a}
T_7 (AS+M)	360.20±12.75^{bc}	361.54±12.79^{bcde}	369.57±13.08^{b}	2.33±0.05^{bc}	2.40±0.06^{abc}	2.23±0.05^{bcd}
T8 (CN+DCD)	311.20±11.51^{e}	261.14±9.66^{f}	319.29±11.81^{c}	2.03±0.05^{efg}	2.12±0.05^{bcdef}	2.02±0.05^{cde}
T9 (CN+M)	323.70±13.20^{cde}	278.16±11.34^{f}	325.71±13.28^{c}	2.03±0.08^{efg}	2.11±0.09^{cdef}	2.07±0.09^{cde}
T10 (AS+CN+DCD)	350.20±18.54^{bcde}	345.31±18.28^{cde}	383.47±20.30^{b}	2.35±0.03^{ab}	2.45±0.09^{abc}	2.38±0.01^{ab}
T11 (AS+CN+M)	357.60±13.38^{bc}	352.61±13.19^{bcde}	381.92±14.29^{b}	2.33±0.02^{bc}	2.35±0.09^{abcd}	2.29±0.09^{abc}
T12 (UR+DCD)	328.40±13.42^{cde}	326.82±13.35^{e}	306.40±12.52^{c}	2.10±0.05^{def}	2.29±0.06^{ab}	2.36±0.06^{ab}
T13 (UR+M)	340.70±13.09^{bcde}	333.92±12.83^{de}	319.92±12.30^{c}	2.27±0.05^{bcd}	2.46±0.10^{bcde}	2.29±0.10^{abc}
SE(d)	19.32	19.04	20.45	0.07	0.12	0.10
LSD (≤0.05)	39.88	40.04	42.22	0.14	0.26	0.21

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.

4.4.3 Enzymatic activities

A perusal of data presented in Table 4.6 revealed that the nitrate reductase activity influenced significantly under different N-sources and nitrification inhibitors. 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. Application of ammonium sulphate registered maximum values of nitrate reductase activity, *i.e.*, 497.02, 478.31 and 469.68 (µmol nitrite formed g^{-1} Fw h^{-1}) during winter, autumn and summer, respectively. Polyphenol oxidase and glutamine synthatase activity were found non-significant during all seasons (Table 4.7).

4.5 Leaf Nutrient Acquisition: Leaf tissue was analysed for macro- (N, P and K) and micro-nutrients (Fe, Cu, Mn and Zn) at 45 days after fertilizer application.

4.5.1 Macro-nutrients

Data pertaining to effect of N-sources and nitrification inhibitors on N, P and K concentration in Kinnow leaves are presented in Table 4.8 and Fig.4.5. The N and K concentration in Kinnow leaves differed markedly with the application of different N-sources and nitrification inhibitors but P remained unaffected. Significantly maximum leaf N (2.90, 3.02 and 2.87%) and K (1.60, 1.67 and 1.62%) were recorded with the application of AS + DCD over control during different three-split application of fertilizers. The application of AS + meliacins, urea + DCD and urea + meliacins remained at par with respect to N and K content by Kinnow leaves during the three season. All the N-sources applied individually remained at par with respect to N and K content by Kinnow leaves. In case of phosphorous, none of the treatments resulted in significant improvement in leaf P content (Fig. 4.5).

4.5.2 Micro-nutrients

Combination of different nitrogen sources and nitrification inhibitors also had pronounced effect on leaf micro-nutrient content of Kinnow (Table 4.9 and Fig. 4.6-4.8). Significantly higher leaf copper content was recorded in AS + DCD (19.00, 19.00 and 18.84 ppm during autumn, spring and summer flushes, respectively) followed by AS + meliacins and urea + DCD, while the lowest values were recorded with control treatment. However, the maximum iron content was noticed in AS + DCD (164.80, 172.03 and 170.85 ppm during autumn, spring and summer flush, respectively)

Table 4.7 Effect of different nitrogen sources with or without nitrification inhibitors on nitrogen and polyphenol oxidase (PPO) (specific activity/ enzyme protein) and glutamine synthatase (GS) (µmoles γ-glutamyl hydroxymate formed g^{-1} FW h^{-1}) content in Kinnow leaves during autumn, spring and summer flushes.

Treatment	PPO			GS		
	Autumn	Spring	Summer	Autumn	Spring	Summer
T_1 (Control)	8.45±0.34	7.83±0.70	9.03±0.41	134.00±5.46	143.11±12.80	135.00±6.07
T_2 (AS)	8.95±0.20	8.78±0.19	8.56±0.34	137.60±3.04	134.16±2.96	140.06±5.54
T_3 (CN)	8.56±0.24	8.63±0.39	8.10±0.23	130.20±5.39	120.38±5.44	134.80±3.77
T_4 (AS+ CN)	8.66±0.22	8.28±0.21	8.65±0.37	135.40±3.40	141.36±3.55	141.22±5.99
T_5 (UR)	8.74±0.26	8.94±0.39	9.49±0.30	136.70±6.24	124.67±5.48	142.40±4.56
T_6 (AS+DCD)	8.30±0.27	8.84±0.30	9.33±0.30	143.00±2.38	146.72±4.97	142.00±2.36
T_7 (AS+M)	9.56±0.22	9.46±0.22	9.14±0.21	140.00±3.23	135.24±3.12	141.19±3.26
T8 (CN+DCD)	8.48±0.21	8.88±0.22	8.58±0.21	134.80±3.27	130.22±3.16	139.00±3.37
T9 (CN+M)	8.75±0.36	8.11±0.33	8.66±0.39	135.00±5.51	144.18±5.88	144.90±6.52
T10 (AS+CN+DCD)	9.00±0.34	9.58±0.34	9.20±0.45	144.80±2.01	148.13±5.29	139.54±4.98
T11 (AS+CN+M)	9.02±0.24	8.96±0.34	9.57±0.36	146.70±2.47	143.03±5.35	134.31±5.02
T12 (UR+DCD)	9.00±0.21	8.94±0.21	8.68±0.30	142.70±3.37	137.42±3.29	145.07±3.43
T13 (UR+M)	9.10±0.19	9.01±0.41	8.12±0.37	143.40±3.07	138.95±6.26	143.05±6.52
SE(d)	0.35	0.51	0.49	5.36	8.22	7.01
LSD (≤0.05)	NS	NS	NS	NS	NS	NS

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 4.8 Effect of different nitrogen sources with or without nitrification inhibitors on nitrogen and potassium (%) content in Kinnow leaves during autumn, spring and summer flushes.

Treatment	Nitrogen (%)			Potassium (%)		
	Autumn	Spring	Summer	Autumn	Spring	Summer
T_1 (Control)	1.62±0.07^{e}	1.88±0.08^{f}	1.65±0.06^{d}	1.28±0.04^{d}	1.25±0.05^{f}	1.26±0.07^{e}
T_2 (AS)	2.45±0.05^{cd}	2.56±0.06^{cde}	2.54±0.05^{c}	1.36±0.04^{cd}	1.36±0.03^{ef}	1.34±0.04^{de}
T_3 (CN)	2.32±0.09^{d}	2.46±0.11^{cde}	2.51±0.10^{c}	1.52±0.07^{abc}	1.50±0.07^{cde}	1.48±0.06^{abcd}
T_4 (AS+ CN)	2.34±0.05^{d}	2.52±0.06^{cde}	2.52±0.05^{c}	1.45±0.06^{abc}	1.47±0.04^{de}	1.45±0.06^{bcd}
T_5 (UR)	2.42±0.11^{d}	2.55±0.10^{cde}	2.48±0.11^{c}	1.37±0.05^{bcd}	1.32±0.07^{f}	1.39±0.05^{cde}
T_6 (AS+DCD)	2.90±0.09^{a}	3.02±0.10^{a}	2.87±0.09^{a}	1.60±0.06^{a}	1.67±0.07^{a}	1.62±0.05^{a}
T_7 (AS+M)	2.88±0.07^{ab}	2.88±0.07^{ab}	2.81±0.06^{ab}	1.59±0.04^{a}	1.65±0.05^{ab}	1.61±0.04^{a}
T8 (CN+DCD)	2.28±0.06^{d}	2.37±0.08^{e}	2.52±0.06^{c}	1.48±0.04^{abc}	1.51±0.04^{bcd}	1.48±0.02^{abcd}
T9 (CN+M)	2.36±0.08^{d}	2.44±0.10^{de}	2.47±0.08^{c}	1.46±0.06^{abc}	1.48±0.02^{de}	1.47±0.06^{abcd}
T10 (AS+CN+DCD)	2.69±0.06^{ab}	2.71±0.09^{bc}	2.67±0.06^{abc}	1.53±0.08^{ab}	1.54±0.03^{abcd}	1.52±0.05^{abc}
T11 (AS+CN+M)	2.67±0.10^{bc}	2.67±0.09^{bcd}	2.62±0.10^{bc}	1.52±0.05^{abc}	1.51±0.06^{bcd}	1.50±0.08^{abc}
T12 (UR+DCD)	2.81±0.07^{ab}	2.86±0.07^{ab}	2.82±0.08^{ab}	1.56±0.04^{a}	1.63±0.04^{abc}	1.56±0.05^{ab}
T13 (UR+M)	2.77±0.10^{ab}	2.84±0.08^{ab}	2.79±0.10^{ab}	1.54±0.07^{ab}	1.56±0.05^{abcd}	1.54±0.04^{abc}
SE(d)	0.11	0.12	0.12	0.08	0.07	0.07
LSD (≤0.05)	0.23	0.26	0.24	0.16	0.15	0.15

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.

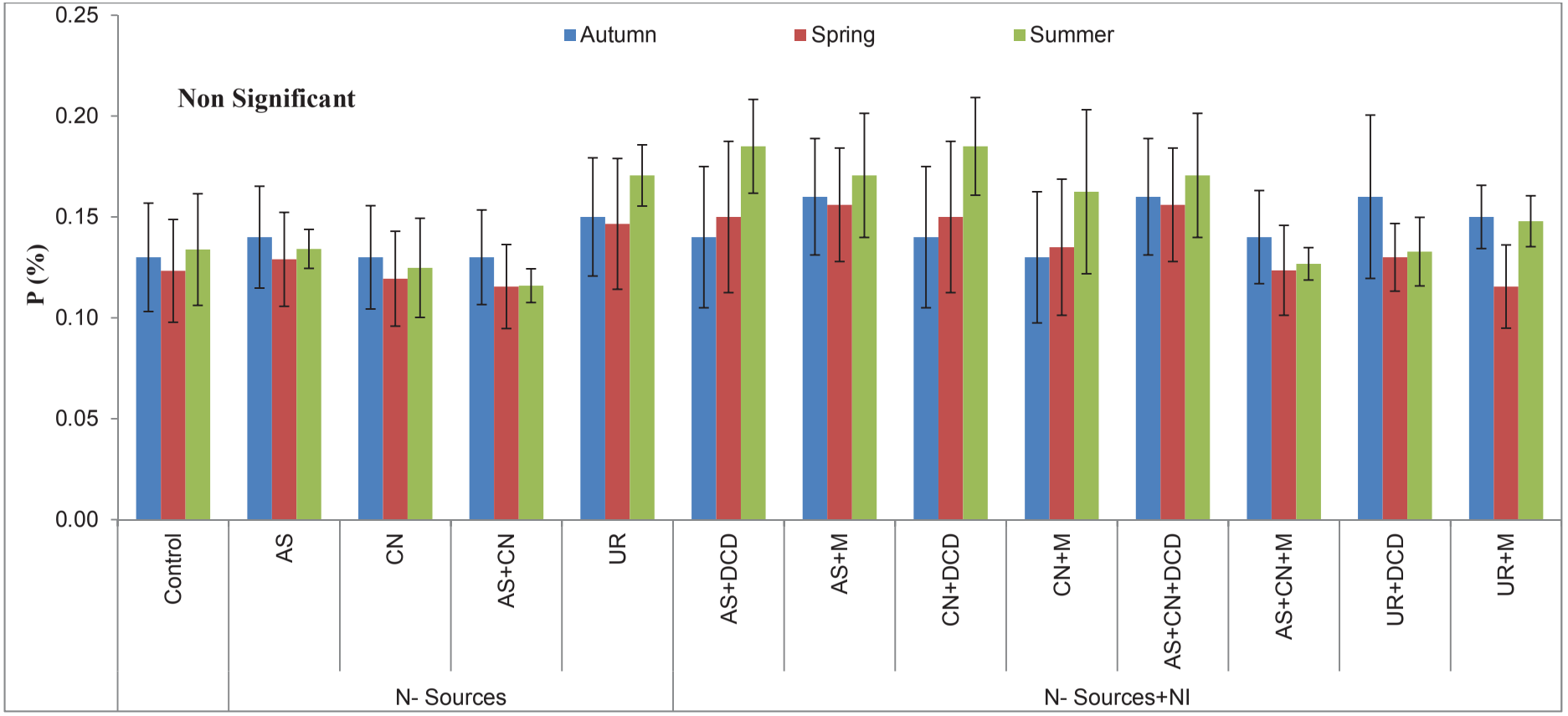

Fig. 4.5 Effect of different nitrogen sources with or without nitrification inhibitors on phosphorous (%) content in Kinnow leaves during autumn, spring and summer flushes. Data represent the mean ± standard error of three independent determinates. Means within same column that did not differ significantly at 5% level of significance when compared with Fisher's Least Significant Difference are followed by the same superscript letters.

Table 4.9 Effect of different nitrogen sources with or without nitrification inhibitors on copper and iron (ppm) content in Kinnow leaves during autumn, spring and summer flushes.

Treatment	Cu (ppm)			Fe (ppm)		
	Autumn	Spring	Summer	Autumn	Spring	Summer
T_1 (Control)	8.00±0.33^{h}	8.20±0.33^{f}	8.80±0.40^{h}	90.00±3.67^{g}	83.43±3.40^{g}	88.22±4.00^{f}
T_2 (AS)	15.70±0.30^{cd}	15.10±0.29^{bcd}	15.28±0.81^{cd}	116.00±2.56^{e}	120.41±2.33^{e}	114.49±6.06^{d}
T_3 (CN)	14.00±0.63^{ef}	12.00±0.54^{e}	11.83±0.53^{g}	101.40±4.58^{fg}	107.90±4.88^{f}	102.21±4.62^{e}
T_4 (AS + CN)	11.20±0.28^{g}	13.80±0.35^{d}	13.39±0.38^{f}	106.60±2.68^{ef}	106.49±6.18^{f}	102.02±2.87^{e}
T_5 (UR)	15.40±0.60^{cde}	14.40±0.56^{cd}	14.01±0.28^{ef}	112.70±4.96^{ef}	114.13±4.45^{ef}	110.29±2.21^{de}
T_6 (AS + DCD)	19.00±0.64^{a}	19.00±0.64^{a}	18.84±0.41^{a}	164.80±5.58^{a}	172.03±5.83^{a}	170.85±3.72^{a}
T_7 (AS + M)	18.20±0.43^{ab}	18.60±0.44^{a}	18.70±0.19^{a}	157.50±3.63^{ab}	161.67±3.82^{ab}	161.69±1.64^{a}
T8 (CN + DCD)	14.40±0.35^{def}	15.00±0.36^{bcd}	14.30±0.29^{def}	107.50±2.61^{ef}	103.84±2.52^{f}	112.55±2.25^{de}
T9 (CN + M)	13.80±0.56^{f}	14.40±0.59^{cd}	13.46±0.36^{f}	112.80±4.60^{ef}	120.47±4.91^{e}	104.57±2.77^{de}
T10 (AS + CN + DCD)	15.00±0.07^{def}	15.80±0.33^{bc}	16.44±0.24^{bc}	135.60±4.84^{cd}	136.68±2.89^{d}	140.20±2.02^{bc}
T11 (AS + CN + M)	17.40±0.59^{b}	15.40±0.52^{bc}	15.27±0.40^{cde}	129.20±4.83^{d}	121.32±4.11^{e}	131.20±3.47^{c}
T12 (UR + DCD)	17.60±0.42^{ab}	17.57±0.42^{a}	17.66±0.64^{ab}	144.50±3.42^{bc}	150.86±3.57^{bc}	143.49±5.17^{b}
T13 (UR + M)	16.80±0.79^{bc}	16.00±0.75^{b}	16.18±0.56^{c}	137.10±6.25^{cd}	143.54±3.64^{cd}	143.54±4.94^{b}
SE(d)	0.73	0.72	0.61	6.3	6.01	5.13
LSD (≤0.05)	1.51	1.48	0.127	13.01	12.42	10.58

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.

followed by AS + meliacins (157.50, 161.67 and 161.69 ppm during autumn, spring and summer flush, respectively), while the minimum levels were observed in control treatment.

A perusal of data on the manganese content in the Kinnow leaves revealed that application of AS + meliacins outclassed all the other treatments and recorded significantly highest content (89.00, 85.40 and 83.95 ppm during autumn, spring and summer flush, respectively). The treatment AS + DCD trailed by urea + meliacins and urea + DCD remained statistically similar in respect to manganese content during the three seasons. When comparison was made between individual N-sources, application of ammonium sulphate, urea and combination of AS + calcium nitrate exhibited higher leaf manganese content than control and calcium nitrate application (Fig. 4.6). Similar results were obtained for leaf Zn content as manganese content (Fig.4.7). The significantly highest zinc content in Kinnow leaf was registered with AS + meliacins (60.40, 66.40 and 63.50 ppm during autumn, spring and summer flush, respectively) than all the other treatments. While the minimum levels were observed in control.

4.6 Rhizospheric soil biological activities: Observations on soil biological activities was measured during the month of October in 2012 (*i.e.,* at the end of experiment) and presented in the Tables 4.10-4.11 and Fig. 4.8.

Significant variation in microbial biomass carbon (MBC) in rhizospheric soil at the end of experiment has been recorded with different N-sources and nitrification inhibitors (Fig. 4.8). The AS + DCD treatment registered significantly highest value (280.31 µg C g^{-1} soil) of MBC, which was found statistically similar to AS + meliacins, urea + DCD, urea + meliacins, AS, AS + CN + DCD and AS + CN + meliacins treatments. Similarly, fluorescein diacetate (FDA) in the rhizosphere soil was also found significantly superior under AS + DCD treatment (4.25 fluorescein µg g^{-1} soil h^{-1}) followed by urea + DCD (3.83 fluorescein µg g^{-1} soil h^{-1}) treatment. Whereas, AS + meliacins, AS + CN + meliacins and urea + DCD treatments remained statistically similar for FDA (Fig. 4.8).

The dehydrogenase activity of rhizospheric soil was found higher in the AS and urea treatment as well as in combination with nitrification inhibitors (Table 4.10). It was found higher in the AS treatment (52.20 µg TPF/g soil/day) however, it remained statistically similar to urea alone and in combination with nitrification inhibitors. The alkaline phosphatase activity was maximum under the urea treatment

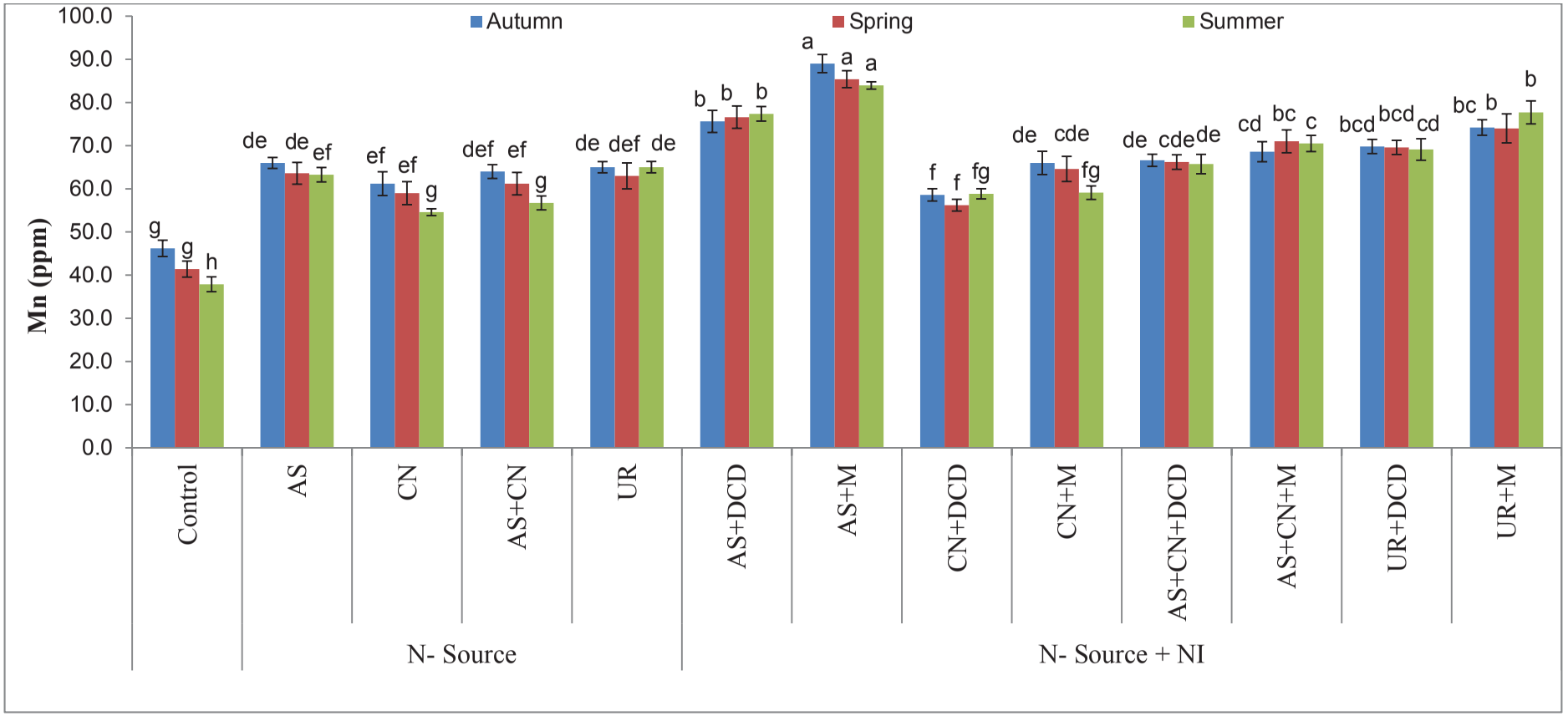

Fig. 4.6 Effect of different nitrogen sources with or without nitrification inhibitors on Mn content (ppm) of Kinnow leaves during autumn, spring and summer flushes. Data represent the mean ± standard error of three independent determinates. Means within same column that did not differ significantly at 5% level of significance when compared with Fisher's Least Significant Difference are followed by the same superscript letters.

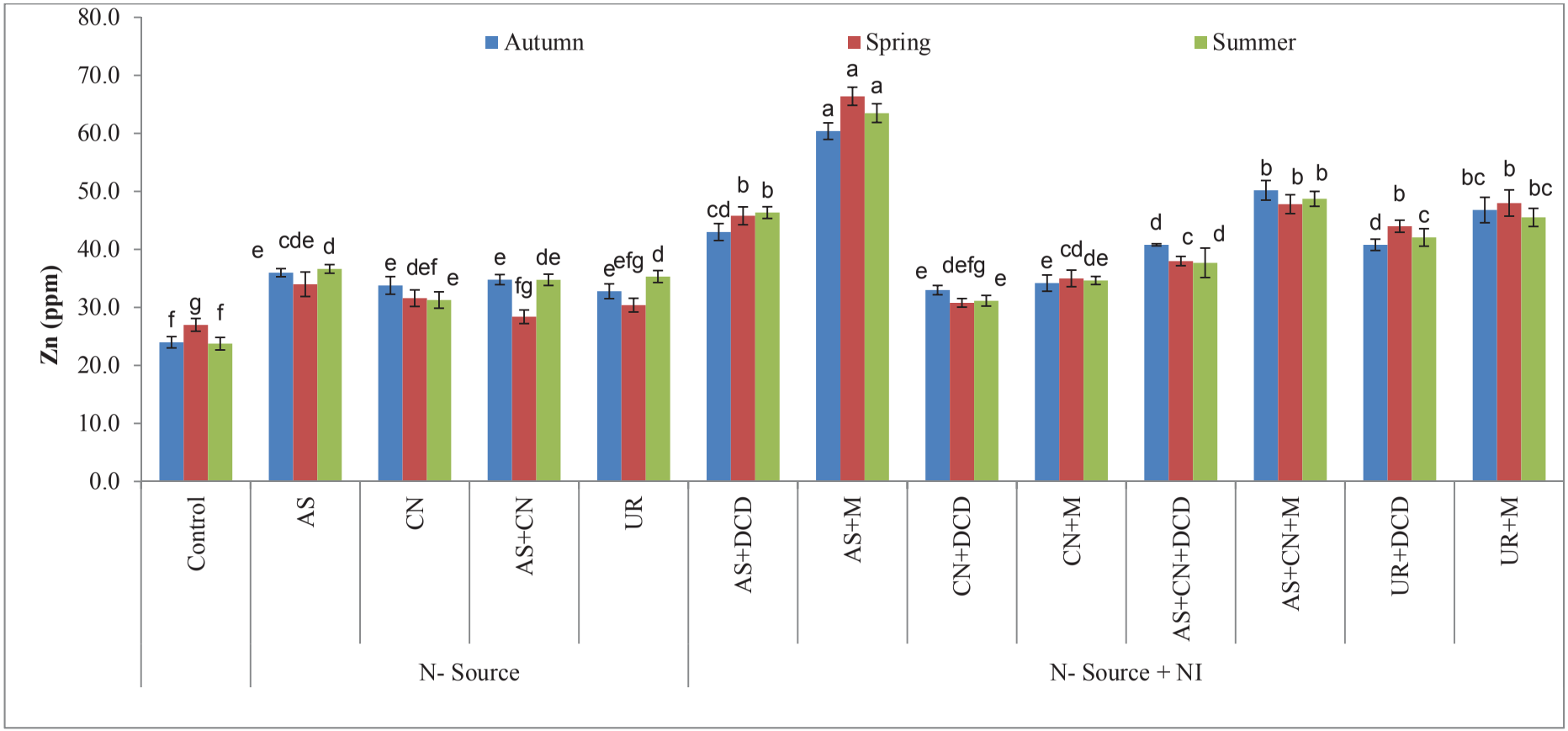

Fig. 4.7 Effect of different nitrogen sources with or without nitrification inhibitors on Zn content (ppm) of Kinnow leaves during autumn, spring and summer flushes. Data represent the mean ± standard error of three independent determinates. Means within same column that did not differ significantly at 5% level of significance when compared with Fisher's Least Significant Difference are followed by the same superscript letters.

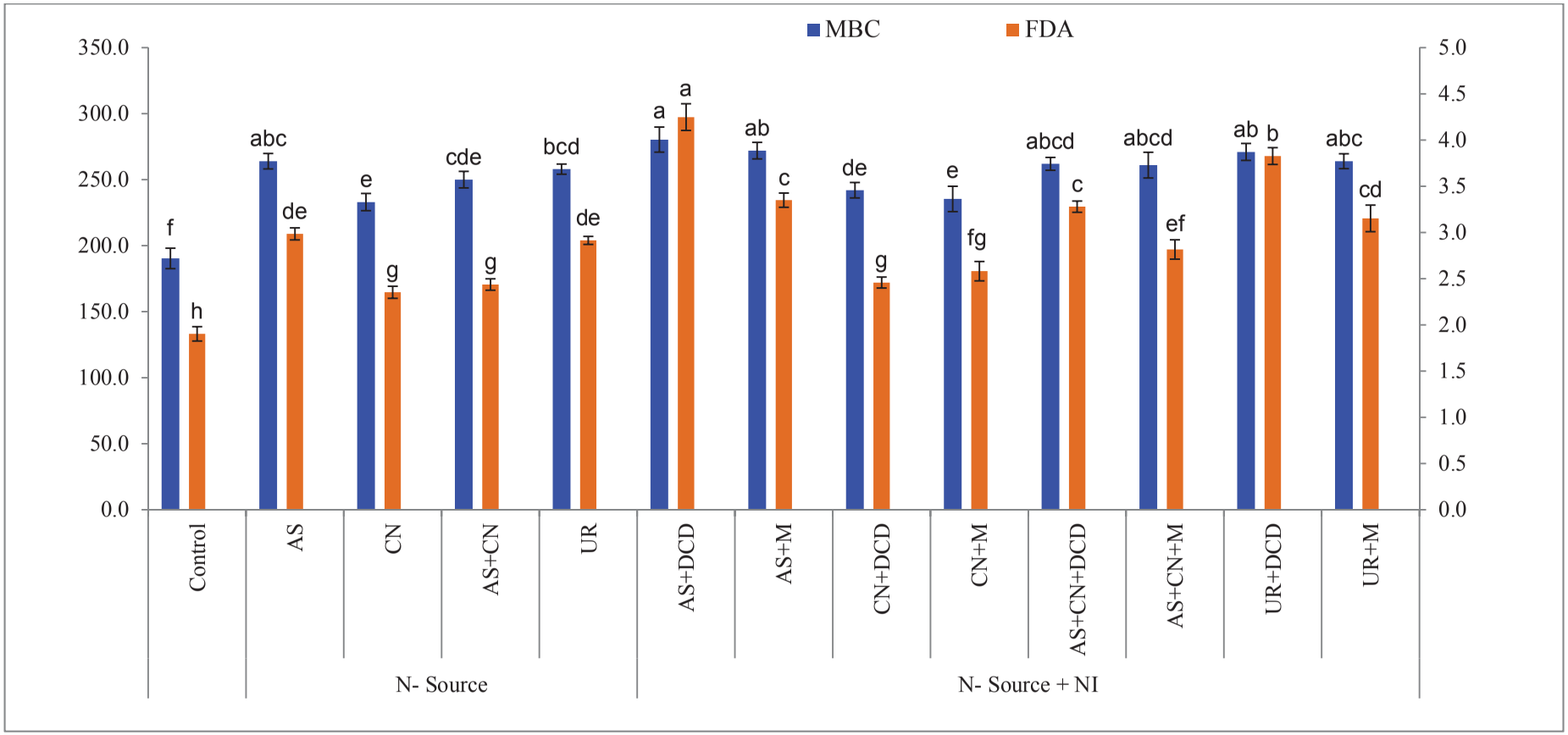

Fig. 4.8 Effect of different nitrogen sources with or without nitrification inhibitors on microbial biomass C (µg C g^{-1} soil) and fluorescein diacetate Assay (fluorescein µg g^{-1} soil h^{-1}) in Kinnow mandarin orchard. Data represent the mean ± standard error of three independent determinates. Means within same 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 4.10 Effect of different nitrogen sources with or without nitrification inhibitors on dehydrogenase (µg TPF/g soil/day) and alkaline phosphatase activity (µg PNP/g soil/h) in Kinnow mandarin.

Treatment	Dehydrogenase activity (µg TPF/g soil/day)	Alkaline phosphatase activity (µg PNP/g soil/h)
T_1 (control)	36.30 ± 1.92^{e}	51.40 ± 2.10^{e}
T_2 (AS)	52.20 ± 1.15^{a}	64.54 ± 1.42^{abcd}
T_3 (CN)	45.20 ± 2.04^{cd}	58.94 ± 2.66^{d}
T_4 (AS + CN)	49.80 ± 0.78^{abc}	68.70 ± 1.73^{ab}
T_5 (UR)	51.20 ± 0.36^{a}	69.24 ± 3.04^{a}
T_6 (AS + DCD)	50.40 ± 2.73^{ab}	68.01 ± 2.30^{abc}
T_7 (AS + M)	50.80 ± 1.28^{ab}	62.60 ± 1.44^{abcd}
T_8 (CN + DCD)	46.30 ± 2.15^{bcd}	58.25 ± 3.60^{de}
T_9 (CN + M)	44.10 ± 0.12^{d}	62.05 ± 2.27^{bcd}
T_{10} (AS + CN + DCD)	49.00 ± 1.66^{abc}	63.32 ± 3.35^{abcd}
T_{11} (AS + CN + M)	48.30 ± 0.39^{abcd}	66.54 ± 2.49^{abc}
T_{12} (UR + DCD)	49.80 ± 2.27^{abc}	61.44 ± 1.45^{cd}
T_{13} (UR + M)	48.80 ± 1.82^{abc}	58.60 ± 2.67^{d}
SEd±	2.25	3.44
LSD (≤0.05)	4.64	7.09

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 4.11 Effect of different nitrogen sources with or without nitrification inhibitors on soil urease activity (µg urea hydrolysed/g soil/h) and β-glucosidase activity (mg PNP kg-1 soil h^{-1}) in Kinnow mandarin.

Treatment	Urease activity (µg urea hydrolysed/g soil/h)	β-glucosidase activity (mg PNP kg-1 soil h^{-1})
T_1 (control)	178.80 ± 7.29^{c}	10.40 ± 0.69
T_2 (AS)	210.30 ± 4.64^{ab}	12.34 ± 0.47
T_3 (CN)	206.30 ± 5.78^{ab}	11.52 ± 0.47
T_4 (AS + CN)	208.30 ± 5.23^{ab}	11.76 ± 0.50
T_5 (UR)	222.30 ± 3.32^{a}	12.28 ± 0.44
T_6 (AS + DCD)	203.50 ± 6.89^{b}	11.41 ± 0.39
T_7 (AS + M)	204.20 ± 4.71^{b}	11.64 ± 0.41
T_8 (CN + DCD)	201.60 ± 4.89^{b}	11.73 ± 0.43
T_9 (CN + M)	202.00 ± 4.75^{b}	11.40 ± 0.46
T_{10} (AS + CN + DCD)	205.10 ± 7.53^{b}	12.80 ± 0.68
T_{11} (AS + CN + M)	205.00 ± 7.67^{b}	11.12 ± 0.42
T_{12} (UR + DCD)	208.10 ± 4.92^{ab}	11.54 ± 0.47
T_{13} (UR + M)	206.80 ± 4.42^{ab}	12.10 ± 0.47
SEd±	8.25	0.68
LSD (≤0.05)	17.03	NS

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

(69.24 µg PNP/g soil/h) and remained statistically similar to AS + CN, AS + DCD, AS + CN + meliacins, AS, AS + CN + DCD and AS + M treatments (Table 4.10). Soil urease activity was found higher with the urea alone (222.30 µg urea hydrolysed/g soil/h) treatment (Table 4.11). Whereas, all the other treatments remained at par except control treatment, whereas, no significant variation was noticed in case of soil β-glucosidase activity due to different N-sources and nitrification inhibitors application (Table 4.11).

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5. Discussion

The research findings of the present investigation entitled **"Effect of nitrogen sources and nitrification inhibitors on nitrogen dynamics and plant performance of Kinnow mandarin"** has been described on nitrogen dynamics in soil, growth parameters, plant physio-biochemical parameters, leaf nutrient status and soil biological activities in the previous chapter. In this chapter, an attempt has been made to analyze the results critically in the light of cause and effect relationship. The findings of earlier workers on the subject have also been taken into consideration while discussing the results of the present investigation.

5.1 Nitrogen dynamics in Kinnow soil

The concentration of NH_4^+-N in soil at 0-30 and 30-60 cm depths below the drippers and 30 cm away from the drippers in all three split applications was significantly higher in ammonium sulphate followed by urea fertilizers treated with DCD and meliacins than they were applied individually (Table 4.1 & 4.2). Inhibitory effect of DCD and meliacins on the nitrification process resulted in more NH_4^+ accumulation in soil. Due to nitrification inhibitors, NO_3^--N availability remains high on surface level (0-30) and reduced NO_3^- leaching in the deeper soil layer (30-60 cm) (Table 4.2). This indicates that the NH_4^+ was nitrified in the soil when amended with N-fertilizers without treating nitrification inhibitors (both DCD and meliacins). Whereas, nitrification inhibitors slow down the nitrification when N-fertilizers treated with these nitrification inhibitors. Thus, the inhibitory effect of DCD and meliacins on the nitrification process resulted in more NH_4^+ accumulation in soil and reduced NO_3^- leaching in the deeper soil layer.

Among N-fertilizers alone, the ammonical N-fertilizer form retained more NH_4^+-N in the upper soil profile (0-30 cm) followed by urea then the other form of N-fertilizers used in this study. When the nitrogen source is in the ammonical form, resistance to leaching occurs due to cationic attraction of ammonium ions by clay and humus (Smith *et al*., 1961; Ramos *et al*., 2013; Jimenez and Lao, 2005). Nitrate ions are highly mobile in the soil, contributing to the contamination of ground waters, can suffer denitrification and accumulate in plant tissues, whereas, the

ammonium ion is not as readily subject to leaching loss. Nitrification converts a relatively immobile NH_4^+-nitrogen (N) to highly mobile NO_3^-N. When DCD and meliacins used with ammonium sources of N, NH_4^+ remained availabile for extended period of time, making it possible to increase NH_4^+ uptake and possibly realized benefits of an enhanced NH_4^+ supply. In a normal sequence of events, N fertilizer is applied to the soil and NH_4^+-N forms are rapidly nitrified to NO_3^--N. This NO_3^- form also leaches readily and may be displaced from the root zone. Studies with nitrification inhibitors have been mainly conducted to keep more N fertilizer in the slowly leachable NH_4^+ form, and thus conserve N for the crop.

In a previous set of experiments in citrus, Serna *et al*. (1994) also observed that DCD was able to delay nitrification, to reduce NO_3^- leaching and to increase N uptake by citrus plants. Preliminary studies carried out in young citrus trees grown in soil culture in pots, revealed a remarkable effect of nitrification inhibitor, 3,4-dimethylpyrazole phosphate (DMPP) on decreasing NO_3^--N levels both in soil and in leaching water, as well as an increase in N uptake of treated plants (Serna *et al*., 2000). Quinones *et al*. (2009) also made similar observations with Clementine cv. Nules mandarin grafted on Troyer citrange (*Citrus sinensis* x *Poncirus trifoliata*) rootstock under field conditions and found that the NH_4^+-N concentration in the 0-20 and 20-40 cm soil layers was significantly higher in the ammonium sulphate (AS) + nitrification inhibitor (NI) treatment. Soil NH_4^+-N contents in N + DCD and N + DCD + S treatments were higher than that of N treatment within 40 days after fertilization in apple orchard (Ge *et al*., 2011). Neem (*Azadirachta indica*) cake was found to inhibit nitrification effectively both in the laboratory and green-house (Sahrawat and Parmar, 1975). Kumar *et al.* (2011) suggested that the coating of prilled urea with meliacins proved to be most effective in enhancing yield and NUE of rice as compared to uncoated prilled urea or coated with other neem oil components. Banuls *et al*. (2001) found that the addition of DMPP to AS resulted in higher levels of NH_4^+-N and lower levels of NO_3^--N in the soil during the whole experimental period to citrus plants. The NO_3^--N concentration in drainage water was lower in the AS plus DMPP- treated pots. Similar results were also found by Martinez-Alcantara *et al*. (2013) during their experiment on citrus.

5.2 Vegetative growth parameters

The continuously higher soil inorganic N contents for the DCD and meliacins treatments were also beneficial for the growth. Therefore, we observed better plant height, spread and shoot growth rate for the DCD and meliacins treatments (Fig. 1 & Table 4.3).

Percentage increase in tree height was significantly higher in AS + DCD followed by AS + meliacins, Urea + meliacins and urea + DCD. Similarly, tree spread E-W, tree spread N-S, specific leaf area and shoot growth rate were found maximum in AS + meliacins. When different N-forms alone (without NI) were compared with control, all the growth parameters were found higher in treatment containing urea and ammonium sulphate.

The better growth with NH_4^+ source might be attributed to availability of higher NH_4^+-N from AS fertilizer which further prolonged with the use of nitrification inhibitors (DCD and meliacins) and consequently resulted in higher uptake of NH_4^+ form by the Kinnow plants. It has earlier been observed that a mixed NH_4^+ and NO_3^- source is preferred, but in some cases, one form is favoured over another (Hageman, 1980; Haynes, 1986). Nitrate uptake is coupled with H^+ uptake, whereas, NH_4^+ is coupled with a change in membrane polarization caused by releasing H^+ into the external medium. Consequently, plants exhibit great differences in their ability to take up and use ammonium and nitrate as sources of N (Haynes and Goh 1978; Haynes, 1986), which reflects the environment to which the species are adapted (Kronzucker *et al*., 1997, 2003; Min *et al*., 1999).

Kinnow and other citrus crops can use both NH_4^+-N and NO_3^--N. Wallace (1954) examined the uptake of ^{15}N-labeled ammonium and nitrate by citrus trees and found that in water culture, Valencia orange cuttings absorbed more ammonium than nitrate. Two reports from Smith (Smith, 1957; Smith and Rasmussen, 1957) showed that citrus seedlings grew normally with exclusive NH_4^+-N as a source provided excess acidity when avoided. Latter, Smith and Rasmussen (1961) concluded that citrus could be grown on a wide range of N sources with equal success. In the long term, ammonium undergoes nitrification to nitrate and the differences between the two kinds of fertilizer would be liable to diminish. In apple there was found more or

less equal absorption from the two kinds of fertilizer, with a slight advantage for ammonium in the winter (Grasmanis and Nicholas, 1971).

Citrus is a perennial tree crop with a growth cycle that comprises three main shoot growth flushes (spring, summer and autumn flush). In citrus trees nitrogen uptake varies greatly over the year (It shows a minimum during dormancy). At the beginning of the cycle, nitrogen demand is very high. However, adverse conditions like soil temperatures are unfavourable for adequate N uptake hence, an intense reserve mobilization takes place to meet the needs of developing organs (Legaz *et al*., 1995; Legaz *et al*., 1982). Nitrogen taken up by roots in spring and summer provides about 20-30% of total leaf N citrus (Feigenbaum *et al*., 1987). There is every reason to believe that where ammonical nitrogen is supplied it is taken up by the plants partially as ammonical and partially as nitrate nitrogen and that the percentage absorption of these forms by the plant will vary considerably from season to season depending upon soil moisture, temperature, pH and other factors at the time or following the fertilizer application (Sites *et al*., 1955). In citrus many studies about the sources of N performed under the acidic soil condition which ultimetly give poor results with respect to ammonical-N due to development of excess acidity in soil (Sites *et al*., 1955; Smith and Rasmussen, 1961; Stewart and Wheaton, 1965; Bown *et al*., 2010). Two reports (Smith, 1957; Smith and Rasmussen, 1957) in 1957 showed that citrus seedlings grew normally with exclusive ammoniacal-N as a source provided excess acidity could be avoided. In our results growth parameters were found higher in treatment containing NH_4^+-N. Khokhar *et al.* (2012) also recommended to add nitrogen as ammonium sulphate, which is less susceptible to leaching and more efficient at sandy and high pH conditions in Kinnow mandarin. Therios and Sakellariadis (1988) observed a higher vegetative growth of olive plants when they were fertilized with ammonium or urea than when nitrate was the N form of fertilization. The similar observations were reported by Garcia *et al*. (1999) when a sterile sand substrate was used to grow olive plants. In short term water culture experiment by Serna *et al*. (1992) with different ^{15}N labeled ammonium or nitrate concentrations, citrus seedlings absorbed higher NH_4^+ at a higher rate than NO_3^- ion.

The treatment AS + DCD and AS + meliacins showed better results for growth parameters as nitrification inhibitors maintained N in the plant's root zone and prevent it from leaching which was reflected in the vegetative growth in the Kinnow

plants. Dutra *et al*. (1994) found 10% more leaf surface, 6% more graft diameter, 5% more graft length and 7.6% more spring knots in controlled-release fertilized Carrizo citrange seedlings (*Citrus sinensis* x *Poncirus trifoliata*) grafted on Hernandina tangerine (*Citrus reticulata* Blanco). Colugnati *et al*. (1997) studied on grapevine and indicated in its results that nitrogen supply by isobutylidene diurea (IBDU) and dicyandiamide (DCD) positively influence total buds formation, sprouting of buds and cluster numbers. The highest plant height of rice was observed in meliacins-coated urea, which was significantly higher than other neem oil components' coated urea (Kumar *et al*., 2011). Increased biomass was observed with a more profuse development of root system when citrus trees fertilized with AS+DMPP (Martinez-Alcantara *et al*., 2013).

5.3 Physiological and Biochemical parameters

The results clearly elucidates that ammonical form of nitrogen with nitrification inhibitors exhibited significant response for physio-biochemical parameters (Table 4.4-4.7 & Fig. 4.2-4.4). Application of AS + DCD registered significantly maximum values of a, b and total chlorophyll content during first, second and third split application. The value of mean photosynthetic rate and stomatal conductance was significantly higher with the treatment AS+DCD, however, it remained statistically similar to AS + meliacins, urea + meliacins and urea + DCD treatment.

Both N forms, ammonium (NH_4^+) and N nitrate (NO_3^-) are important N sources for plant growth (Marschner, 1995), but they act differently on the physiological and metabolic processes of plants (Helali *et al*., 2010) and thus influencing plant development substantially (Jimenez and Lao, 2005). Nitrogen is the primary nutrient element needed in the greatest quantities for plant growth and development. Nitrogen is a constituent of proteins, amino acids, RNA, DNA, and many other essential molecules in plants. Perhaps the greatest impact that N has on plant nutrition is in relation to photosynthesis. A largest portion of N allocated to leaves is invested in ribulose 1,5-bisphosphate carboxylase-oxygenase (RUBISCO). Nitrogen is also a necessary component of other enzymes involved in carbon assimilation reactions of photosynthesis and in the light-energy harvesting machinery of the pigment complexes (Lawlor, 1994).

Additionally, the source of N can have a strong impact on the energy demand for N uptake, transport and incorporation into plant proteins. Ammonium is converted into glutamine and glutamate in energy-requiring reactions catalyzed by the enzymes glutamine synthetase and glutamate synthase (Stewart *et al*., 1980). Nitrate must be converted into nitrite and then NH_4^+ in energy-requiring reactions catalyzed by nitrate reductase and nitrite reductase before it can be assimilated into amino acids. Therefore, NO_3^- requires more energy for assimilation than does NH_4^+. It has been suggested that continued input of N into the plant is responsible for the maintenance of leaf and better photosynthetic activity. A higher N supply in leaves should allow a more appropriate redistribution of N to support growth and higher level of N in the leaves to maintain the photosynthetic apparatus. The increased chlorophyll content with N addition as AS and urea with nitrification inhibitors indicated that more N is allocated to the light-harvesting complex. By contrast, the increase in photosynthesis indicated that more N may be allocated to the enzymes of the carbon reactions. Thus, more N was presumably allocated to RUBISCO and the other enzymes of the carbon reactions, more CO_2 could be used by the plant. Apparently, N was invested in the proteins and chlorophyll of the photosynthetic apparatus (Foyer *et al*., 2001). 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. It is also reported in foliage plants in which one of the fundamental qualities is leaf colour. The use of ammonium fertilizers and the addition of nitrification inhibitors to ammonium fertilizers led to a greener leaf colour, probably due to an increase of the chlorophyll content in NH_4^+-N fed plants (Bonasia *et al*., 2008) and to an increase of iron concentration in leaf (Ramos *et al*., 2013).

Ammonium sulphate treated with DCD produced statistically the highest total soluble sugars, soluble proteins and free amino acids during winter, autumn and summer, respectively followed by ammonium sulphate treated with meliacins.

Plants supplied with ammonium contain high levels of free ammonium, amide, glucosamine, and free basic amino acids, such as lysine and arginine, in comparison with nitrate-treated plants (Harada *et al.,* 1968; Takaki *et al.,* 1968). Increases in amino acids and amines in ammonium-fed plants are the result of the detoxification

of ammonia by amino-acid synthesis with organic acids as the carbon source. Unlike nitrate, ammonia requires no reduction; but it is toxic, and so must be combined with a non-nitrogenous compound in order to synthesize harmless and useful nitrogenous constituents such as amino acids, amines or guanidino compounds. Increase in carbohydrate concentration in tomato plants under NH_4^+-N fertilization in comparison to NO_3^--N (Horchani *et al*., 2010). The nitrate reductase activity influenced significantly under different N-sources and nitrification inhibitors. Application of ammonium sulphate registered the maximum values of nitrate reductase activities. The ammonium sulphate and urea treated with nitrification inhibitors showed lower nitrate reductase activity due to lower substrate (NO_3^-) availability than both the untreated fertilizers. Some studies suggest that high ammonium concentration will reduce nitrate reductase activity in shoots and roots and therefore inhibit nitrate uptake (Haynes and Goh, 1978; Downs *et al*., 1993; Sagi and Lips, 1998). Several workers like Flaig and Mohr (1992) and Bown *et al*., 2010 found that ammonium and nitrate were taken up at the same rate in seedlings of *Pinus* as if they were applied separately suggesting that ammonium does not inhibit nitrate uptake at least in long-term studies than those involved in uptake kinetics.

5.4 Leaf nutrient acquisition

Maintaining leaf nutrient concentrations at an optimal level is one of the key issues for profitable citrus orchards. When N is in short supply, growth and yield are limited and the foliage becomes pale green or yellow.

Nutrient composition (N, P, K, Zn, Cu, Mn and Fe) of leaves varied due to application of differential response to different nitrogen sources and nitrification inhibitors. However, the amount of N, P and K in leaves was adequate in all the treatments comprising NI when compared to the foliar diagnostic chart developed for optimum Kinnow mandarin productivity (2.28-2.53% N, 0.10-0.13% P and 1.28-1.63% K) for North Indian condition (Hundal and Arora, 2001). The micronutrient concentration in the leaves was also affected significantly due to different nitrogen sources and nitrification inhibitors treatments.

Significantly maximum leaf N and K were recorded with the application of AS + DCD over control during all three split application of fertilizers. The application of AS + meliacins, urea + DCD and urea + meliacins remained at par with respect to N

and K content in Kinnow leaves irrespective of seasons. Significantly higher leaf copper and iron content was recorded in AS + DCD, However, significantly maximum manganese and zinc content was found in AS + meliacins treatment during autumn, spring and summer flushes (Table 4.8-4.9 and Fig.4.5-4.8).

Application of nitrification inhibitors resulted in availability of both the form of N at the surface level and it might be reflected in the leaf N of Kinnow plants. In previous studies the addition of DCD to ammonium sulphate nitrate resulted in a significant increase in leaf N concentration in citrus (Serna *et al*., 1994, 1996). Banuls *et al*. (2001) found higher concentration of nitrogen in the leaves and roots of citrus with the application of ammonium sulfate (AS) + 3,4-dimethylpyrazole phosphate (DMPP) than with AS alone. The decrease in NO_3^- leaching as a result of DCD application also led to reduced leaching losses of cations, *e.g.,* Ca_2^+, K^+ and Mg^{2+}, due to reduced requirement of counter ions for NO_3^- (Di and Cameron, 2004b). Quinones *et al*. (2009) was carried out an experiment with Clementine cv. Nules mandarin grafted on Troyer citrange rootstock under field conditions and found that the addition of NI to AS originated a significantly higher N and Fe concentrations in the spring-flush leaves. A nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) supplementation results in less K leached significantly when ammonium sulphate nitrate (ASN) ($(NH_4)_2SO_4$ and NH_4NO_3) are supplied as the nitrogen form in the sandy loam soil due to lack of NO_3^- as the counter ions (Wu *et al*., 2007). Kumar *et al*. (2007) obtained the higher concentration of nitrogen in grain and straw of rice coupled with higher dry matter yields resulted in higher total N uptake with meliacins-coated urea.

Alternatively, the manipulation of nitrogen (N) nutrition can have a significant effect on the retranslocation of Fe and Zn. Nitrogen nutrition is assumed to have a positive effect, because N is required for the biosynthesis of chelator nicotianamine (NA) and the Fe transport peptide ITP (Kruger *et al*., 2002; Shi *et al*., 2012). In most agricultural soils, nitrate (NO_3^-) and ammonium (NH_4^+) are the most abundant inorganic N forms. Besides serving as N source, these two N forms do also distinctly affect many physiological processes such as changes in rhizosphere pH or in the synthesis of organic acids which, in turn, may act as micronutrient chelators (Zou *et al*., 2001; Marschner, 2012). It is, therefore, Khokhar *et al.* (2012) recommended to

add nitrogen as ammonium sulphate, which is less susceptible to leaching and more efficient at sandy and high pH conditions.

Serna *et al*. (1992) who found a higher foliar N, P, Mg, Fe and Cu concentration in citrus fed with ammonium in comparison with nitrate. In addition, micronutrients deficiency occurs in trees growing in such alkaline soils (Amberger, 1982). In this respect, Johnston (2004) reported that when ammonium sulphate is applied, one pH unit can be decreased and this pH change is important for P availability supply and may be also for micronutrient availability. In a recent study, it has been reported that compared to nitrate, the supply of ammonium to hydroponically-grown wheat plants resulted in a general increase in nutrient concentrations, including Fe, Cu as well as Zn, and particularly bioavailablity of Zn (Carlisle *et al*., 2012). These results are likely related to the fact that nitrate and ammonium can cause distinct or even antagonistic effects on the pH of the rhizosphere soil and the apoplast of root or leaf cells as well as on the uptake, translocation and remobilization of micronutrients (Zou *et al*., 2001; Marschner, 2012).

Zinc deficiency is prevalent worldwide in temperate and tropical climates (Fageria *et al*., 2003, Slaton *et al*., 2005). It is especially widespread in calcareous soils with high pH. An analysis of soil samples taken from different states showed that 47% of Indian soils are deficient in Zn (Takkar 1996). Zinc deficiency is also widespread in citrus trees in India and has resulted in low fertility status of Kinnow orchards (Khokhar *et al*., 2012). Srivasatava and Singh (2004) mentioned that in soils with very high pH, availability of Zn^{++} to plant roots is extremely low. The increase in soil pH is associated with increased absorption of Zn^{++} to soil hydroxides, carbonates and organic matter and decreased absorption by plant roots.

DCD is used to enhance solubility of micronutrients and relatively small amounts of DCD are capable of increasing the micronutrient solubility. DCD increases the solubility of many trace nutrients in soil considerably and stabilizes for long time.

5.5 Soil biological parameters

Different nitrogen sources alone and in combination with nitrification inhibitors differed significantly with respect to soil biological activities (Fig. 4.8 &

Table 4.10-4.11). The AS + DCD treatment registered significantly highest value of microbial biomass carbon and fluorescein diacetate. The dehydrogenase and alkaline phosphatase activity of rhizospheric soil was found higher in the AS and urea treatment as well as in combination with nitrification inhibitors.

NH_4^+ is the preferred N source for ectomycorrhizal fungi in pure culture although many species also utilize NO_3^- if NH_4^+ supply is limited (France and Reid, 1983). Therefore, in the root region, we would expect NH_4^+ to be preferred to NO_3^- if the concentration of the ions was similar at the absorbing surfaces of microbes or roots. Microbial preference for NH_4^+ over NO_3^- has been reported by some researchers (Jansson, 1958; Azam *et al*., 1993). Mahmood *et al.* (2005) found that ammonium compared to nitrate nutrition of wheat and maize had significantly increased microbial biomass in the rhizosphere soil. They used dicyandiamide as nitrification inhibitor to maintain ammonium as the predominant N source for plants grown in ammonium treated soil.

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6. Summary and Conclusion

6.1 Summary

The present study entitled "Effect of nitrogen sources and nitrification inhibitors on nitrogen dynamics and plant performance of Kinnow mandarin" was carried out at the Division of Fruits and Horticultural Technology, along with facilities available from Plant Physiology, Agricultural Chemicals, Soil Science and Agricultural Chemistry and Water Science and Technology, IARI New Delhi during 2011-2012. The present experiment was carried out with the objectives to find out the effect of different nitrogen sources and nitrification inhibitors on nitrogen dynamics in soil, vegetative growth, physiological processes and biochemical constituents of Kinnow plants and changes in soil biological activities. There were 13 treatments comprising four nitrogen sources (ammonium sulphate, calcium nitrate, mixture of ammonium sulphate + calcium nitrate and urea), two nitrification inhibitor (dicyandiamide @ 5% of N-fertilizers, meliacin @ 0.1% of N-fertilizers) and control. The salient findings of present investigation are summarized below:

Experiment I: Effect of different nitrogen sources and nitrification inhibitors on soil nitrogen distribution in Kinnow orchard

- The concentration of NH_4^+-N in soil at 0-30 cm (44.1, 55.7, 42.7 mg kg^{-1} soil below drippers and 37.8, 41.9, 36.0 mg kg^{-1} soil at 30 cm away from drippers) and 30-60 cm depth (24.2, 20.2, 23.2 mg kg^{-1} soil below drippers and 20.0, 23.5, 23.6 mg kg^{-1} soil at 30 cm away from drippers) in all three split applications respectively, was significantly higher when ammonium sulphate followed by urea treated with DCD than they were applied alone.
- The NO_3^--N concentrations in soil at 0-30 cm depth below were significantly higher in the AS + DCD treatment (33.9, 41.0, 31.4 mg NO_3^--N kg^{-1} soil below drippers and 27.3, 37.0, 31.1 mg NO_3^--N kg^{-1} soil at 30 cm away from drippers in all three split applications respectively) followed by urea + DCD and AS + meliacins treatments.
- The lowest NO_3^--N was estimated in soil at 30-60 cm depth with AS + DCD (18.8, 20.5, 19.1 mg NO_3^--N kg^{-1} soil below drippers and 16.8, 18.3, 19.9 mg NO_3^--N kg^{-1} soil at 30 cm away from drippers in all three split applications respectively) followed by AS + meliacins and AS + DCD treatment as most

of the NO_3^- N remained in the upper soil layer (0-30 cm depth) due to slow nitrification.

Experiment II: Influence of nitrogen sources and nitrification inhibitors on vegetative growth, physiological processes and biochemical constituents in Kinnow plant

- Control of nitrification by DCD and meliacins lead to increased efficiency of ammonium sulphate and urea fertilizers with corresponding improvement in plant growth rate (per cent increase in tree height, spread, shoot lengths and specific leaf area).
- Percentage increase in tree height was significantly higher in 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^{-1}) and shoot growth rate (247.39%) was found maximum in AS + meliacins.
- 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 mg g^{-1} Fw chl a, chl b and total chlorophyll content during first, second and third split application, respectively).
- Among the untreated N-fertilizers, AS followed by urea showed higher photosynthetic rate, stomatal conductance and transpiration rate.
- The value of mean photosynthetic rate (5.06, 5.95 and 5.71 µmol m^{-2} s^{-1} during first, second and third split application, respectively) and stomatal conductance (37.52, 38.41 and 36.82 mmol m^{-2} s^{-1} during first, second and third split application, respectively) was significantly higher with the treatment AS+DCD, however, it remained statistically similar to AS + meliacins, urea + meliacins and urea + DCD treatment.
- Application of AS + meliacins registered significantly maximum values for transpiration rate, *i.e.*, 2.15 and 2.13 mmol m^{-2} s^{-1} during winter and summer split application respectively, whereas, in winter (second split) it was found higher (2.35 mmol m^{-2} s^{-1}) under AS + DCD treatment.
- Ammonium sulphate treated with DCD produced statistically the highest total soluble sugars (9.22, 9.78 and 9.40 % leaf fresh wt) and soluble proteins (74.80, 76.49 and 71.96 mg g^{-1} leaf dry wt) during winter, autumn and summer, respectively followed by ammonium sulphate treated with meliacins.

- Application of ammonium sulphate registered the maximum values of nitrate reductase activities, *i.e.*, 497.02, 478.31 and 469.68 (µmol nitrite formed g^{-1} fw h^{-1}) during winter, autumn and summer, respectively due to higher substrate (NO_3^-) availability under this treatment.
- Significantly maximum leaf N (2.90, 3.02 and 2.87%) and K (1.60, 1.67 and 1.62%) were recorded with the application of AS + DCD over control during all three split application of fertilizers. The application of AS + meliacins, urea + DCD and urea + meliacins remained at par with respect to N and K uptake in Kinnow leaves irrespective of seasons.
- Significantly higher leaf copper (19.00, 19.00 and 18.84 ppm) and iron (164.80, 172.03 and 170.85 ppm) content was recorded in AS + DCD, However, significantly maximum manganese (89.00, 85.40 and 83.95 ppm) and zinc content (60.40, 66.40 and 63.50 ppm) was found in AS + meliacins treatment during autumn, spring and summer flushes, respectively.

Experiment III: Determination of soil biological activities with respect to nitrogen sources and nitrification inhibitors under Kinnow plants

- Significant beneficial variation in soil biological activities at the end of experiment has been recorded with different N-sources and nitrification inhibitors.
- The AS + DCD treatment registered significantly the highest value of MBC (280.31 µg C g^{-1} soil) and fluorescein diacetate (4.25 fluorescein µg g^{-1} soil hr^{-1}).
- The dehydrogenase and alkaline phosphatase activities of rhizospheric soil was found higher in the AS and urea treatment as well as in combination with nitrification inhibitors treatments.

6.2 Conclusion

The investigation clearly brought out the fact that the addition of the Dicyandiamide (5% of N-fertilizers) and meliacins (0.1% of N-fertilizers) to NH_4^+ containing N sources reduced NO_3^- leaching and increase N fertilizer utilization efficiency in Kinnow production under high soil pH conditions. The source of nitrogen has a strong impact on growth and physio-biochemical parameters in Kinnow plants. Additionally, higher soil inorganic N contents for the DCD and meliacins treatments were also beneficial for the growth and provided higher N, K

and micro-nutrients in the leaves to support growth and to maintain the photosynthetic activity in Kinnow mandarin plants for better plant health and performance under alkaline soil pH conditions. Integrated use of Dicyandiamide (5% of N-fertilizers) and meliacins (0.1% of N-fertilizers) with ammonium sulphate and urea had pronounced influence on soil health parameters, *viz.*, status of available N, K and micro-nutrients, activities of soil enzymes (dehydrogenase, alkaline phosphatase, fluorescein diacetate and urease) and microbial biomass carbon. The results strongly recommend advantageous use of Dicyandiamide (5% of N-fertilizers) and meliacins (0.1% of N-fertilizers) with ammonium sulphate and urea for a more efficient development of Kinnow plants under alkaline soil pH conditions.

⁂

Abstract

Effect of nitrogen sources and nitrification inhibitors on nitrogen dynamics and plant performance of Kinnow mandarin

A field experiment on two-year-old Kinnow / *Jatti khatti* plants was carried out for three seasons (2011-12) at the Todapur Orchard of Division of Fruits and Horticultural Technology, Indian Agricultural Research Institute, New Delhi. 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 soil of the experimental site was sandy loam in texture with pH 8.2 and EC 0.19 dS/m in the top 0-30 cm of soil and pH 7.8 and EC 0.15 dS/m in the top 30-60 cm of soil. The treatments comprised 4 nitrogen sources (ammonium sulphate as ammonical form, calcium nitrate as nitrate form, mixture of ammonium sulphate + calcium nitrate and urea), two nitrification inhibitors (Dicyandiamide (DCD) @ 5% of N-fertilizers and meliacin @ 0.1% of N-fertilizers) and one control. There were 13 treatment combinations [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 + DCD, T_7 = ammonium sulphate + meliacins, T_8 = calcium nitrate + DCD, T_9 = calcium nitrate + meliacins, T_{10} = ammonium sulphate + calcium nitrate + DCD, T_{11} = ammonium sulphate + calcium nitrate + meliacins, T_{12} = urea + DCD and T_{13} = urea + meliacins]. Recommended fertilizers dose was applied in three splits, *i.e.*, during Winter season in September (75 g N : 37.5 g P : 52.5 g K plant^{-1}), during Spring season in February (150g N : 75g P : 105 g K plant^{-1}) and during Rainy season in June (75 g N : 37.5 g P : 52.5 g K plant^{-1}). Nitrification inhibitor was mixed with different nitrogenous fertilizers before application and then applied in the rhizospheric soil. The experiment was laid out in randomized block design and replicated thrice. Experimental orchard was installed with online drip irrigation system.

The inhibitory effect of DCD and meliacins on the nitrification process resulted in more accumulation of NH_4^+ and least leaching of NO_3^- in soil. The concentration of NH_4^+-N in soil at 0-30 (44.1, 55.7, 42.7 mg kg^{-1} soil below drippers and 37.8, 41.9, 36.0 mg kg^{-1} soil at 30 cm away from drippers) and 30-60 cm depth (24.2, 20.2, 23.2 mg kg^{-1} soil below drippers and 20.0, 23.5, 23.6 mg kg^{-1} soil at 30 cm away from drippers) in all three split applications respectively, was significantly higher in ammonium sulphate + DCD treatment followed by urea +DCD treatment than they were applied individually. Due to application of nitrification inhibitors, NO_3^--N

availability was high in the surface level, reduced NO_3^- leaching and better N fertilizer utilization efficiency in Kinnow production. The NO_3^--N concentrations at 0-30 cm depth were significantly higher in the AS + DCD treatment (33.9, 41.0, 31.4 mg NO_3^--N kg^{-1} soil below drippers and 27.3, 37.0, 31.1 mg NO_3^--N kg^{-1} soil at 30 cm away from drippers), whereas, the concentration of NO_3^- N in soil at 30-60 cm depth was found lower in treatment the AS + DCD (18.8, 20.5, 19.1 mg NO_3^--N kg^{-1} soil below drippers and 16.8, 18.3, 19.9 mg NO_3^--N kg^{-1} soil at 30 cm away from drippers) in all three split applications, respectively.

Increase in tree height was found significantly higher in AS + DCD (44.05%) treatment, whereas, tree spread E-W (77.33%), tree spread N-S (66.03%), specific leaf area (123.86 cm^2 g^{-1}) and shoot growth rate (247.39%) were found maximum in AS + meliacins. Application of AS + DCD registered significantly maximum values of a, b and total chlorophyll content, photosynthetic rate and stomatal conductance, however, transpiration rate was maximum under treatment AS + meliacins during winter and summer split application of fertilizers. Ammonium sulphate treated with DCD produced statistically the 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^{-1} leaf dry wt) during winter, spring and summer, respectively followed by ammonium sulphate treated with meliacins. Significantly maximum leaf N (2.90, 3.02 and 2.87) and K (1.60, 1.67 and 1.62) were recorded with the application of AS + DCD over control during all three split application of fertilizers. Significantly higher leaf copper (19.00, 19.00 and 18.84 ppm) and iron (164.80, 172.03 and 170.85 ppm) content were recorded in AS + DCD, However, significantly maximum manganese (89.00, 85.40 and 83.95 ppm) and zinc (60.40, 66.40 and 63.50 ppm) content were found in AS + meliacins treatment during autumn, spring and summer flush, respectively.

The AS + DCD treatment registered significantly highest value of microbial biomass carbon (280.31 µg C g^{-1} soil) and fluorescein diacetate (4.25 fluorescein µg g^{-1} soil hr^{-1}). The dehydrogenase and alkaline phosphatase activity of rhizospheric soil was found higher in the AS and urea treatment as well as in combination with nitrification inhibitors.

From the findings of this experiment, it can be concluded that the addition of the nitrification inhibitor to ammonium sulphate and urea reduces NO_3^- leaching and increase N utilization efficiency in Kinnow performance.

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सारांश

नत्रजन स्त्रोतों एवं नाइट्रीकरण संदमकों का नत्रजन-गतिशीलता और किन्नों पादप प्रदर्शन पर प्रभाव

द्विवर्षीय किन्नों/जत्ती खट्टी पौधों पर तीन ऋतुओं (2011–12) हेतु, भारतीय कृषि अनुसंधान संस्थान, नई दिल्ली में फल एवं औद्यानिक प्रौद्योगिकी संभाग के टोडापुर उद्यान में एक प्रक्षेत्र प्रयोग किया गया। दिल्ली की जलवायु, गर्म शुष्क ग्रीष्मकाल एवं ठंडे शीतकाल सहित अर्ध–शुष्क, उपोष्ण कटिबंधी श्रेणी में आती है और यह कृषि–पारिस्थितिक क्षेत्र–IV के अन्तर्गत आती है। प्रायोगिक स्थल की मृदा, 0–30 सेमी की ऊपरी मृदा पी एच मान 8.2 एवं ई सी 0.19 डी.एस./मी. तथा ऊपर से 30–60 से.मी. मृदा पी एच मान 7.8 एवं ई सी 0.15 डी.एस./मी. के साथ कणाकार में बलुई दोमट थी। किए गए उपचारों में 4 नाइट्रोजन स्रोतों (अमोनिकल अवस्था के रूप में अमोनियम सल्फेट, नाइट्रोजन अवस्था के रूप में कैल्शियम नाइट्रेट, अमोनियम सल्फेट + कैल्शियम नाइट्रेट तथा यूरिया), दो नाइट्रीकरण संदमकों {एन–उर्वरकों के 5 प्रतिशत की दर से डाइसायनडाइएमाइड (डी.सी.डी.) एवं नत्रजन उर्वरकों के 0.1 प्रतिशत की दर से मेलियासिन} तथा एक कंट्रोल का समावेश था। उपचार–संयोजनों की संख्या 13 थी {($टी_1$ = नियंत्रित भूखंड, $टी_2$ = अमोनियम सल्फेट (ए.एस.), $टी_3$ = कैल्शियम नाइट्रेट (सी.एन.), $टी_4$ = अमोनियम सल्फेट + कैल्शियम नाइट्रेट, $टी_5$ = यूरिया (यू.आर.), $टी_6$ = अमोनियम सल्फेट + डी.सी.डी., $टी_7$ = अमोनियम सल्फेट + मेलियासिन्स, $टी_8$ = कैल्शियम नाइट्रेट + डी.सी.डी., $टी_9$ = केल्शियम नाइट्रेट + मेलियासिन्स, $टी_{10}$ = अमोनियम सल्फेट + कैल्शियम नाइट्रेट + डी.सी.डी., $टी_{11}$ = अमोनियम सल्फेट + कैल्शियम नाइट्रेट + मेलियासिन्स, $टी_{12}$ = यूरिया + डी.सी.डी. एवं $टी_{13}$ = यूरिया + मेलियासिन्स)}। संस्तुत उर्वरकों की खुराक का अनुप्रयोग तीन भागों में किया गया, अर्थात, शीतकाल ऋतु के दौरान सितम्बर में (75 ग्राम नत्रजन : 37.5 ग्राम फॉस्फोरस : 52.5 ग्राम पोटेशियम प्रति पौंधा), वसन्त ऋतु के दौरान फरवरी में (150 ग्राम नत्रजन : 75 ग्राम फॉस्फोरस : 105 ग्राम पोटेशियम प्रति पौंधा) एवं वर्षा ऋतु के दौरान जून में (75 ग्राम नत्रजन : 37.5 ग्राम पोटेशियम प्रति पौंधा)। अनुप्रयोग से पहले नाइट्रीकरण संदमक को विभिन्न नाइट्रोजनयुक्त उर्वरकों के साथ मिश्रित किया गया और तत्पश्चात मूलपरिवेशी मृदा में अनुप्रयोग किया गया। यह प्रयोग यादृच्छिक भूखंड डिज़ायन में तथा तीन प्रतिकृतियों के साथ किया गया। प्रायोगिक उद्यान में ऑनलाइन बूँद–बूँद सिंचाई तंत्र स्थापित था।

नाइट्रीकरण प्रक्रिया पर डी सी डी एवं मेलियासिन्स के संदमनी प्रभाव के परिणामस्वरूप मृदा में अमोनियम का अधिक संचयन हुआ और अमोनिया का न्यूनतम क्षरण हुआ। मृदा में 0–30 से.मी. गहराई पर अमोनिकल नत्रजन की सांद्रता (ड्रिपर्स के नीचे 44.1, 55.7, 42.7 मिली ग्राम प्रति किलोग्राम मृदा तथा ड्रिपर्स से 30 से.मी. दूर 37.8, 41.9, 36.0 मि.ली. ग्राम प्रति किलोग्राम मृदा) तथा 30–60 से.मी. गहराई (ड्रिपर्स के नीचे 24.2, 20.2, 23.2 मि.ली. ग्राम प्रति किलोग्राम मृदा तथा ड्रिपर्स से 30 से.मी. दूर 20.0, 23.5, 23.6 मि.ली. ग्राम प्रति किलोग्राम मृदा) पर क्रमशः सभी तीनों विभक्त अनुप्रयोगों में, अमोनियम सल्फेट + डी.सी.डी. एवं उसके बाद यूरिया + डी.सी.डी. अनुप्रयोग के अन्तर्गत उनके अलग–अलग प्रयोग की तुलना में सार्थक रुप से अधिक थी। नाइट्रीकरण संदमकों के

अनुप्रयोग के कारण मृदा सतह सतर में नाइट्रेट नत्रजन की उपलब्धता में वृद्धि हुई, नाइट्रेट नत्रजन के क्षरण में कमी आयी तथा एन–उर्वरक उपयोग क्षमता बेहतर थी। सभी तीनों विभक्त अनुप्रयोगों में, ए एस + डी सी डी उपचार (ड्रिपर्स के नीचे की मृदा में 33.9, 41.0, 31.4 मिली ग्राम नाइट्रेट नत्रजन प्रति किलोग्राम तथा ड्रिपर्स से 30 से.मी. दूर 27.3, 37.0, 31.1 मिली ग्राम नाइट्रेट नत्रजन प्रति किलोग्राम), में 0–30 से.मी. गहराई पर नाइट्रेट नत्रजन सान्द्रताएं महत्वपूर्ण रूप से अधिक थीं जबकि उपचार ए.एस. + डी.सी.डी. (ड्रिपर्स के नीचे 18.8, 20.5, 19.1 मिली ग्राम नाइट्रेट नत्रजन प्रति किलोग्राम मृदा तथा ड्रिपर्स से 30 से.मी. दूर 16.8, 18.3, 19.9 मिली ग्राम नाइट्रेट नत्रजन प्रति किलोग्राम मृदा) में 30–60 से.मी. गहराई पर मृदा में नाइट्रेट नत्रजन की सान्द्रता कम पायी गईं।

वृक्ष की ऊँचाई में बढ़ोतरी, ए.एस. + डी.सी.डी. उपचार में महत्वपूर्ण रूप से अधिक (44.05 प्रतिशत) पायी गई, जबकि ए.एस. + मेलियासिन्स उपचार में वृक्ष का फैलाव पूर्व–पश्चिम (77.33 प्रतिशत), वृक्ष का फैलाव उत्तर–दक्षिण (66.03 प्रतिशत), विशिष्ट पर्ण क्षेत्रफल 123.86 वर्ग से.मी. प्रति ग्राम एवं प्ररोह वृद्धि दर (247.39 प्रतिशत) अधिकतम पाए गए। उर्वरकों के शीतकालीन एवं ग्रीष्मकालीन विभक्त अनुप्रयोग के दौरान, ए.एस. + डी.सी.डी. के अनुप्रयोग से ए, बी एवं कुल पर्णहरित अंश, प्रकाश संश्लेषण दर एवं पर्ण रंध्रो संबंधी चालकता के महत्वपूर्ण रूप से अधिकतम मान प्राप्त हुए, वैसे ए.एस. + मेलियासिन्स उपचार के अन्तर्गत वाष्पोर्त्सजन दर अधिकतम पायी गई। उर्वरकों के सभी तीनों विभक्त अनुप्रयोगों के दौरान नियंत्रित भूखंड की तुलना में डी.सी.डी. के साथ उपचारित अमोनियम सल्फेट द्वारा क्रमशः सर्दी, वसंत एवं गर्मी के दौरान सांख्यिकीय रूप से अधिकतम कुल घुलनशील शर्करा (9.22, 9.78 एवं 9.40 प्रतिशत पत्ती ताजा भार) एवं घुलनशील प्रोटीनों (74.80, 76.49 एवं 71.96 मिली ग्राम प्रति ग्राम पर्ण शुष्क भार) प्राप्त हुए जिसके पश्चात मेलयासिन्स के साथ उपचारित अमोनियम सल्फेट का स्थान रहा। उर्वरकों के सभी तीनों विभक्त अनुप्रयोगों के दौरान, नियंत्रित भूखंड की तुलना में ए.एस. + डी.सी.डी. के अनुप्रयोग द्वारा महत्वपूर्ण रूप से अधिकतम पर्ण नत्रजन (2.90, 3.02 एवं 2.87 प्रतिशत) एवं पोटेशियम (1.60, 1.67 एवं 1.62 प्रतिशत) दर्ज किये गए। ए.एस. + डी.सी.डी. में महत्वपूर्ण रूप से अधिक पर्ण कॉपर (19.00, 19.00 एवं 18.84 पी.पी.एम.) अंश दर्ज किए गए, वैसे, ए.एस. + मेलियासिन्स उपचार में क्रमशः ऑटम (गर्मी एवं सर्दी के बीच का समय), वसंत एवं समर फ्लश के दौरान, महत्वपूर्ण रूप से अधिकतम मैंगनीज़ (89.00, 85.40 एवं 83.95 पी.पी.एम.) एवं जिंक (60.40, 66.40 एवं 63.50 पी.पी.एम.) अंश पाए गए।

ए.एस. + डी.सी.डी. उपचार में सूक्ष्मजीव जैवमात्रा कार्बन (280.31 माइक्रो ग्राम कार्बन प्रति ग्राम मृदा) एवं फ्लोरेसिन डाइएसिटेट (4.25 फ्लोरेसिन माइक्रो ग्राम प्रति ग्राम मृदा प्रति घंटा) के महत्वपूर्ण रूप से अधिक मान दर्ज किए गए। ए.एस. एवं यूरिया उपचार तथा साथ ही नाइट्रीकरण संदमकों के साथ संयोजन में मूल परिवेशी मृदा की डीहाइड्रोजिनेज़ एवं एल्केलाइन फॉस्फेटेज़ सक्रिया उच्चतर पायी गई।

इस प्रयोग के परिणामों से यह निष्कर्ष निकाला जा सकता है कि अमोनियम सल्फेट एवं यूरिया के साथ नाइट्रीकरण संदमको का योजन नाइट्रेट नत्रजन के क्षरण को कम करता है और किन्नों में नत्रजन उपयोग क्षमता में बढ़ोतरी करता है।

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*Original not seen

⁂

APPENDIX-I

Mean weekly meteorological data during 2011-12.

Month	Std. week	Rainfall (mm)	Tmax (^{0}C)	Tmin (^{0}C)	Sunshine (h)	Rhmean (%)	Evaporation (mm)
September	36	113.8	32.5	24.4	3.0	90	2.4
September	37	38.2	33.0	25.5	5.2	84	3.2
September	38	11.6	32.9	24.0	7.8	81	3.3
September	39	0.0	34.3	22.7	9.2	68	4.9
October	40	0.0	34.3	21.6	9.0	65	5.0
October	41	0.0	34.0	19.8	7.7	62	5.0
October	42	0.0	33.1	15.8	7.8	55	4.2
October	43	0.0	31.3	14.7	4.1	57	3.5
November	44	0.0	30.9	13.4	5.3	57	3.3
November	45	0.0	30.5	14.7	5.0	59	3.3
November	46	0.0	29.1	12.8	6.6	58	3.2
November	47	0.0	27.3	12.0	1.8	69	2.2
November	48	0.0	25.6	9.8	2.8	61	2.8
December	49	0.0	27.1	11.9	4.4	69	2.1
December	50	0.0	22.0	5.7	3.9	63	2.6
December	51	0.0	21.0	2.8	2.4	66	2.1
December	52	0.0	20.1	1.7	1.9	71	1.7
January	1	6.6	19.5	8.8	0.0	85	1.5
January	2	0.0	17.5	3.7	3.6	66	1.8
January	3	8.2	17.4	5.6	2.0	77	2.3
January	4	0.0	19.6	5.3	6.5	63	2.4
February	5	0.0	21.0	4.6	6.8	65	3.1
February	6	0.0	20.4	6.3	6.1	55	3.7
February	7	0.0	21.7	8.2	5.4	53	4.3
February	8	0.0	25.7	9.8	7.1	55	4.4
February	9	0.0	25.4	8.3	8.4	50	5.5
March	10	0.0	28.5	11.3	6.1	51	5.6
March	11	19.2	27.1	10.5	7.1	55	4.2
March	12	0.0	31.5	14.9	6.5	46	5.5
March	13	0.0	33.6	16.1	6.7	50	7.1
April	14	0.0	36.6	19.0	8.0	47	7.8
April	15	6.8	34.5	19.8	6.8	61	6.7
April	16	0.0	34.2	19.4	6.1	58	6.0
April	17	2.2	37.5	19.0	8.4	52.9	7.5
may	18	0	35.7	19.7	8.7	43.1	8.3
May	19	0	40.1	24.2	7.2	42.0	9.2
May	20	0	39.3	23.6	5.4	42.1	8.2
May	21	0	42.9	25.6	8.9	34.3	11.0
Jun	22	0	44.2	26.8	9.0	22.6	11.1
JUn	23	12.4	41.3	26.7	6.3	37.1	8.9
Jun	24	0	43.1	29.1	8.2	32.6	11.3
Jun	25	0	42.8	29.8	7.7	36.3	10.3
Jun	26	0	42.3	29.8	5.8	39.9	9.6
July	27	46.0	41.1	27.7	6.0	62	9.2
July	28	63.4	33.0	27.8	3.4	79	4.7
July	29	0.0	37.4	28.2	7.0	62	5.6
July	30	0.0	35.5	27.5	3.2	69	6.0
August	31	54.0	33.4	26.3	1.4	80	3.7
August	32	22.4	33.2	26.2	1.5	83	3.2
August	33	33.8	33.1	25.5	5.1	78	4.5
August	34	80.6	31.8	26.0	1.4	85	3.7
August	35	113.6	31.8	24.6	1.3	84	4.6
September	36	6.4	33.4	25.6	4.9	76	4.8
September	37	32.0	33.8	26.0	4.2	75	4.1
September	38	18.6	32.1	23.4	5.3	74	3.6
September	39	0.0	34.0	21.3	9.5	66	5.2

APPENDIX - II

Initial and final plant spread and height (m) of Kinnow mandarin.

Treatment	Plant spred (m)				Plant height (m)	
	E-W		N-S			
	Initial	Final	Initial	Final	Initial	Final
T1 (Control)	1.54	2.09	1.52	2.02	1.94	2.23
T2 (AS)	1.44	2.22	1.52	2.39	1.84	2.37
T3 (CN)	1.57	2.35	1.52	2.22	1.92	2.41
T4 (AS+ CN)	1.57	2.34	1.64	2.37	1.99	2.50
T5 (UR)	1.40	2.10	1.41	2.25	1.85	2.41
T6 (AS+DCD)	1.54	2.54	1.55	2.51	1.81	2.60
T7 (AS+M)	1.45	2.51	1.50	2.49	1.86	2.66
T8 (CN+DCD)	1.45	2.12	1.55	2.24	1.72	2.14
T9 (CN+M)	1.52	2.19	1.54	2.27	1.87	2.36
T10(AS+CN+DCD)	1.60	2.44	1.63	2.38	1.89	2.44
T11 (AS+ CN+M)	1.55	2.42	1.56	2.30	1.69	2.27
T12 (UR+DCD)	1.62	2.50	1.55	2.34	1.94	2.65
T13 (UR+M)	1.56	2.46	1.48	2.40	1.74	2.49