Impact of Nano-urea on Morpho-physiological, Biochemical and Yield Parameters of Kale (*Brassica oleracea* var. *acephala*)

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Thesis

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DEDICATED TO MY PARENTS AND MY GRANDPARENTS

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
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Certificate – I

This is to certify that the thesis entitled **"Impact of Nano-urea on Morpho-physiological, Biochemical and Yield Parameters of Kale (*Brassica oleracea* var. *acephala*)"**submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science in Plant Physiology**, to the Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, is a record of bonafide research work carried out by **Ms. Muzain Mushtaq (Regd. No. MSBS-2020-18)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that any help or information received during the course of investigation have duly been acknowledged.

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This is to certify that the thesis, **"Impact of Nano-urea on Morpho-physiological, Biochemical and Yield Parameters of Kale (*Brassica oleracea* var. *acephala*)"**submitted by **Ms. Muzain Mushtaq (Regd. No. MSBS-2020-18)** to the Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, in partial fulfilment of the requirements for the award of the degree of **Master of Science in Plant Physiology** was examined and approved by her Advisory Committee and external examiner on ………………..

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ABSTRACT

The present investigation entitled **"Impact of Nano-urea on Morpho-physiological, Biochemical and Yield Parameters of Kale (*Brassica oleracea* var. *acephala*)"** was carried out in the laboratory of Division of Basic Sciences & Humanities, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar during the year 2021-2022. The experiment consisted of 8 treatments, the first treatment (T_1) being the recommended dose of urea, T_2: No urea (water spray only), T_3: urea spray (2000 ppm), T_4: nano-urea spray (2000 ppm), T_5: nano-urea spray (1000 ppm), T_6: nano-urea spray (500 ppm), T_7: nano-urea spray (250 ppm) and T_8: nano-urea spray (125 ppm). These treatments were sprayed 15, 30 and 45 days after sowing. These factors were tested in a completely randomized design with three replications per treatment. Observations on plant growth parameters, physiological parameters, biochemical parameters, quality parameters, molecular parameters, quality parameters and yield parameters were recorded. All the plant growth parameters i.e. plant height (88.50 cm for Khanyari and 48.20 for G M Dari), number of leaves (13.40 for Khanyari and 11.40 for G M Dari), leaf area (1910.63 cm^2 for Khanyari and 1703.97 cm^2 for G

M Dari), fresh weight of leaves(109.20g/plant for Khanyari and 100.20g/plant for G M Dari), and dry weight of leaves(14.70g/plant for Khanyari and 13.20g/plant for G M Dari) for both the varieties were found to be maximum with the foliar application of T_8 treatment (125 ppm nano-urea) i.e, 3.125 mL L^{-1}. The physiological parameters *viz*, net photosynthetic rate (17.20µmole $m^{-2}sec^{-1}$ in Khanyari and 15.40 µmole $m^{-2}sec^{-1}$ in G M Dari), stomatal conductance (0.51 mmole $m^{-2}sec^{-1}$ in Khanyari and 0.40 mmole $m^{-2}sec^{-1}$ in G M Dari) and transpiration rate (5.71 µmole $m^{-2}sec^{-1}$ in Khanyari and 4.70 µmole $m^{-2}sec^{-1}$ in G M Dari) were also found to be maximum with the foliar application of 125 ppm nano-urea (T_8). The net assimilation rate and relative growth rate were also found to be maximum with the foliar application of lowest concentration of nano-urea i.e. 125 ppm. The biochemical parameters like total phenols (2.06 mg GAE g^{-1} in Khanyari and 1.42 mg GAE g^{-1} in GM Dari) and total carbohydrates (5.64 g/100g in Khanyari and 5.06 g/100g in G M Dari) were found to be maximum with the foliar application of 125 ppm of nano-urea (T_8). The quality parameters like vitamin C content (150.20 g/100 g in Khanyari and 148.30 g/100g in G M Dari) and total antioxidant activity (56.30 mg GAE g^{-1} in Khanyari and 45.60 mg GAE g^{-1} in G M Dari) were found to decrease gradually with increasing doses of nano-urea, however their maximum values were observed with the application of T_8 treatment i.e. 125 ppm of nano-urea. Some parameters like leaf chlorophyll a content, leaf chlorophyll b content , total leaf chlorophyll content, leaf carotenoid content, leaf nitrate content, leaf N , P , K , Ca, leaf crude protein and soluble protein content were found to be maximum with the foliar application of highest concentration of nano-urea (i.e. 2000 ppm), but because this concentration was too high (excessive), plant development and all other quality metrics were reduced. At this concentration, nitrate content accumulated mostly in the leaves. The yield parameters like yield per plant (104.10 g/plant in Khanyari and 85.10 g/plant in G M Dari) and yield per hectare (34.50 t ha^{-1} in Khanyari and 27.10 t ha^{-1}) were also found to be maximum for T_8 treatment i.e. 125 ppm nano-urea. The lowest yields were obtained in control. The nano application proved practical and more efficient since a concentration as low as 125 ppm of nano-urea proved superior to that of 2000 ppm ordinary urea.

Keywords: Biochemical parameters, Foliar application, Kale, Nano-urea, Quality, Yield

Signature of Student **Signature of Major Advisor**

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MUZAIN MUSHTAQ

Place: **Shalimar, Srinagar**
Dated:

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Chapter -1

INTRODUCTION

Kale (*Brassica oleracea* var. *acephala*) is one of the oldest forms of cabbage family and is probably the first brassicas to be cultivated and are quite similar to wild cabbage. It is a green leafy vegetable in which central leaves do not form a head. It belongs to a group of non-heading plants of cabbage family and are hardiest of cole crops. It is biennial or perennial usually grown as an annual crop for its edible leaves. Kale can withstand drought and temperatures of -10 to -15°C (Ahluwalia *et al.* 1979) and therefore, when supply of most of the vegetables is scarce, kales are still conveniently available as greens. Acikgoz (2011) reported that kale has originated from eastern Mediterranean region and is being used as a food crop since 2000 BC. It is a highly nutritious vegetable, rich in vitamins particularly vitamin C, pro-vitamin (β-carotene and lutein) and minerals such as calcium, phosphorus, potassium, magnesium, iron etc. Kale contains highest concentrations of total antioxidants (Cao *et al.*1996). Among cole crops, kale has high amounts of total nitrogen, raw protein, fat, carbohydrate and amino acids. Kales are also rich in leucine, isoleucine, arginine, phenyladenine, lysine and also good amounts of threonine, tryptophan and histidine are also present in it. Kales have a milder and less tangy taste than turnip greens. The retention of vitamin C is highest in kale on cooking. The total sugar content and reducing sugars are higher in kale than cauliflower stalks and cabbage and as such cooked product is comparatively more palatable and delicious (Ahluwalia *et al.* 1979). In Asaba, Delta State of Nigeria, kale is nicknamed "hospital too far" because of its health benefits. It is often called "borecoles" which is taken from Dutch word "Borenkool" and the plant is also called as peasants cabbage in Holland and Germany (Oldham, 1999). It is one of the richest source of carotenoids especially xanthophylls as reported by Ligor and Buszewski (2012). Among worlds 100 healthiest foods, kale grabs the first position in terms of lutein-containing food in the USDA's National Nutrient

Database that analyzes 5, 350 foods that contain this carotenoid nutrient. Sikora *et al.* (2007) reported that kale is a potent source of glucosinolates and contains about 15-20 different glucosinolate like compounds.

Amongst the leafy vegetables kale is cultivated on a large scale in the temperate regions of the world. In India, kale has not been grown as a vegetable crop for commercial use. However it is commercially grown in Kashmir and to a limited extent in Jammu, Assam and Himachal Pradesh. In Jammu and Kashmir it is a popular vegetable grown in almost all kitchen gardens and also as a commercial crop around cities and towns. Kales grown in Kashmir are popularly known as *Hak*. In Kashmir, kale is grown round the year but it shines most as a cold weather crop and it is at its tender and most flavourful condition during winter months. Next to chillies, kale occupies largest area and ranks first in production in Kashmir valley among other vegetable crops. The area under this crop in Kashmir valley is nearly 2300 ha with an annual production of 1,61,00 tonnes (Anonymous, 2000). As reported by Wanchoo (2000) the nomenclature for all edible green leaves has been changed from *Shak* in Sanskrit to *Sag* (present day local nomenclature) in most parts of the country and *Hak* in Kashmir.

Cabbage and other brassicas are at present grown extensively throughout the world over an area of 2470.27 thousand hectares with a productivity of 29.05 tonnes/hectare. In India, kale is being grown over an area of 400.14 thousand hectares with a productivity of 22.59 tonnes/hectare (Anonymous, 2014a). In J& K, it is being grown over an area of 2460 hectares out of which in Jammu region it occupies an area of 160 hectares with a productivity of 18.78 tonnes/hectare (Anonymous, 2014b)

Kale is a heavy feeder of nitrogen and therefore, good nitrogen source is of paramount importance to optimize its economic yield (Onyango *et al.* 2012). However, soil fertility is decreasing progressively due to the imbalanced use of inorganic fertilizer and loss of nutrients from the soil. In the tropical countries like Ethiopia, high cost of fertilizers, scarcity, nutrient imbalance in soil and soil acidity are problems associated with the use of inorganic fertilizers while bulkiness, low nutrient quality and late mineralization are the bottleneck to the sole use organic manures for crop production (Uyovbisere *et al.* 2000). Moreover, alternative soil nutrient sources are expensive for small farmers and the poor timing and awareness of application for improved productivity of kale (Onwonga *et al.* 2013). Therefore there is an urgent need to identify and investigate a cheap, easily available, environmentally friendly source of fertilizer which would enhance balanced supply of crop nutrition, sustainable nutrient availability and thereby maximize the yield of leafy vegetables. As kale is grown commercially and almost in every kitchen garden in Kashmir valley so different approaches should be adopted to increase the quality and yield of this crop. One such approach is the use of nano-fertilizer in place of commercial fertilizer which promotes sustainable agriculture, increases the tolerance of plants against biotic and abiotic stresses, increases the nutrient use efficiency (NUE) and the overall productivity of agricultural systems (Seleiman *et al.* 2020).

A nano fertilizer is defined as a product present in nano meter regime that delivers nutrient to the crops. These are nutrient carriers of nano-dimensions ranging from 10 to 400 nm and are capable of holding nutrient ions due to their high surface area and releases it slowly to correspond with the crop demand. There are slow-release and super sorbent nitrogenous and phosphatic fertilizers (Lal, 2008; Hasaneen *et al.* 2015). Nano fertilizers can possibly enter the plant cells directly through the sieve-like cell wall structures. Nano fertilizers dissolve in solution and are capable of releasing the nutrients as soluble ions. Plants absorb the soluble nutrient ions as quickly as they take in those from dissolved conventional fertilizers. Nano fertilizers have controlled release of nutrients, reduction in toxicity, site targeted delivery and enhanced nutrient utilization of delivered fertilizers (Cui *et al.* 2010).

Nanotechnology applications in agriculture are gradually transforming the theoretical possibilities into practical applications. Nanotechnology possesses the potential to augment agricultural productivity through genetic improvement of plants and animals (Kuzma, 2006; Scott 2007) along with cellular level delivery of genes and drug molecules to particular sites in plants and animals (Maysinger, 2007). Use of nano fertilizers not only reduces environmental pollution, eutrophication, pollution of ground water and diseases caused by overusing of conventional fertilizers, but also due to smaller particle diameters, more penetration into the roots and leaves of plants can improve the physiological traits and yield of crops. Nano fertilizers improve the crop productivity by enhancing the rate of seed germination, seedling growth, photosynthetic activity, nitrogen metabolism, carbohydrate and protein synthesis. However, as being an infant technology, the ethical and safety issues surrounding the use of nanoparticles in plant productivity are limitless and must be very carefully evaluated before adapting the use of nano fertilizers in agricultural fields (Priyanka Solanki *et al.* 2015). Therefore, it is recommended to replace nano fertilizers with conventional fertilizers, especially in sandy soils due to the possibility of more leaching of conventional urea fertilizer which results in ground water pollution (Naimeh Astaneh *et al.* 2021). Mir *et al.*(2018) reported that there is a need to mitigate the hazardous impacts on the environment induced by chemical fertilizers particularly NPK fertilizers. They are applied in huge amounts with low efficiency (N 30-35%, P 18-20% and K 35-40%) causing groundwater pollution by accumulating in soil body.

Experiments on fertilizer efficiency of nano-fertilizers were conducted on many crops like raddish, cabbage, eggplant, peppers, tomatoes, celery and leek (Liu *et al.* 2009). Excessive and improper usage of nitrogen fertilizer causes problems in human health and environment. So there is a need for a suitable alternative source of nitrogen with reduced harm on environment. Nano nitrogen fertilizers are alternative to conventional fertilizers with slow and control release of nitrogen (Rathnayaka *et al.* 2018). Nano-urea increased the agronomic efficiency of nitrogen fertilization by 44.5% and the grain yield by 10.2%, in comparison to normal urea (Shiwen Huang. 2015).

One such effort has been made by IFFCO towards the manufacturing of nano-urea in liquid form and it is the first developed liquid based urea fertilizer in nano form to address the imbalanced and excessive use of conventional urea. Nano-urea (liquid) reduces the requirement of conventional urea by 50% or more. Efficacy of one bottle of nano-urea (500mL) is equivalent to one bag of urea. When sprayed on leaves nano-urea easily enters through stomata and other openings and is assimilated by the plant cells. It is easily distributed through phloem from source to sink inside the plant as per its need. Nano-urea (liquid) does not involve any government subsidy and will be made available to farmers at a 10% lower price than a bag of subsidized urea. Transportation would be easier and economical, as one 500mL bottle would be equivalent to one bag of regular urea fertilizer. One bottle (500 mL) contains 40,000 parts per million (ppm) of nitrogen which is equivalent to impact of nitrogen nutrient provided by one bag of conventional urea weighing 50 Kg. Furthermore, apart from improving yield, soil health and nutritional quality of crop, nano-urea has also been tested for biosafety and toxicity according to norms followed in India and the international guidelines developed by OECD, which are globally adopted and accepted. Nano-urea has been included in the Government's Fertiliser Control Order after the field trials were undertaken under National Agriculture Research System (NARS), 20 ICAR Research Institutes, State Agriculture Universities and Krishi Vighyana Kendras on 43 crops (Prem Baboo, 2021).

Effects of nano-fertilizers have been observed on various crops and leafy vegetables but there is hardly any literature available pertaining to effect of nano-urea on kale (*Brassica oleracea* var. *acephala*). Hence the present study was conducted with the following objectives:

1. To evaluate the effect of nano-urea spray on physiological and biochemical parameters of kale

2. To determine the optimum dose of nano-urea for improving the quality and yield of kale

Chapter 2

REVIEW OF LITERATURE

A comprehensive review of literature is of great importance for any research and is an integral part of investigation as it not only gives an idea on the work done in the past and assists in delineation of the problem but also provides bases for interpretation of results and discussion. A brief review of the earlier studies conducted on the subject is presented in this chapter under the following headings.

2.1 Impact of nano nitrogen fertilizer on growth and yield attributes

2.2 Nanoparticle delivery in the plant system

2.3 Effect of nanofertilizers on yield and yield component

2.1 Impact of nano nitrogen fertilizer on growth and yield attributes

Amirnia *et al.* (2014) reported that in saffron nano-fertilizers improved saffron yield and it was also clear that Fe, P and K nano-fertilizers all had positive effects on flowering. In total it was concluded that environmental conditions, farm management, correct choice of saffron ecotypes, mother corm weight and nano-fertilizers are important factors in economic saffron production.

Manikandan *et al.* (2015) reported that in maize grain N content of nanozeourea on inceptisol and alfisols were higher consistently. The growth, yield, quality and nutrient uptake were consistently higher for nanozeourea treatment than conventional urea.

Abdel-Salam (2018) reported that nano technique in foliar spray of urea proved extremely efficient in increasing growth parameters of lettuce. A foliar spray using as little concentration as 2500 mg nano-urea NL^{-1} proved superior to that of 5000 mg NL^{-1} ordinary urea N. A foliar spray with 3750 mg nano-urea NL^{1} increased growth parameters by surpassing those given by the 5000 mg NL^{-1} soluble urea by upto 100% or more. Application of nano-urea must not exceed

3750 mg NL^{-1}, otherwise a decrease in growth would occur at the excessive concentration of 5000 mg nano-urea NL^{-1}.

Mijweil *et al.* (2018) reported that in potato, among the various fertilizer combinations, fertilizer + nano-fertilizer recommendation yielded the best results in most of the vegetative qualities, quantity and quality of the tubers compared to other fertilizer combinations.

Merghany *et al.* (2019) reported that nano-fertilizer treatments significantly improved the plant growth, yield and fruit quality of cucumber and it can be used as an alternative to mineral fertilizers.

Rani *et al.* (2019) reported that in sorghum RDN was reduced 2.5 times through nano-fertilizer application and there was a significant increase in growth parameters, yield and quality characters of sorghum crop. This study clearly indicated that the application of nano-fertilizer can save about 40% dose of recommended nitrogenous fertilizer in sorghum crop.

Rop *et al.* (2019) reported that in maize, capsicum and kale, SRF showed increased maize grain yield, capsicum fruit numbers and increased kale dry matter and yield.

Shams (2019) reported that in Kohlrabi (*Brassica oleracea* var. *gongylodes*) spraying plants with 100mg N L^{-1} nano- chitosan- urea together with plant inoculation with AMF is the recommended solution to cut off the inputs of chemical N fertilizers by 33.3%.

Vassell *et al.* (2019) reported that in kale the length of the roots and shoots were positively affected when its seeds were treated with aqueous solutions of CuO NPs. There was an increase in the dry weight of kale plants. This study showed that application of CuO NPs at appropriate concentrations causes increase in the growth and development of kale.

Babita Mishra *et al.* (2020) reported that in tomato, application of 50% N+ 100% PK+ 50% Zn inorganically to soil along with first foliar spray with nano nitrogen, second spray with nano Zn and third spray with nano Cu was found best in producing higher plant height, more number of branches, fruit length, fruit girth, fruit weight, number of fruits per plant and also higher yield.

Mohammadi *et al.* (2020) reported that the application of nano-nitrogen fertilizer had significant effects on reducing nitrate leaching and increasing sugar production in sugarcane.

Naim *et al.* (2020) reported that in oyster mushroom the re-use of spent oyster substrate may provide a nutritious, low- cost substrate with a potential to produce comparable yield to that obtained in conventionally used wheat straw. Added benefits may be acquired from such substrate when treated with nano-urea, mostly twice during the production cycle. The study provided evidences on shortening in periods between consecutive flushes and improvement in biological yield, depending upon the applied dose of nano-urea.

Yogendra Kumar *et al.* (2020) reported that in winter season crops of Rajasthan, nanofertilizers are considered as a novel approach towards saving of nutrients, in particular nitrogen. With the application of nanofertilizers, the nutrient use efficiency can be significantly enhanced as revealed by 50% saving of urea through two sprays of nano nitrogen.

Reddy *et al.* (2021) reported that in maize, combined application of 50% N through urea + 50% N through nano-urea combined with nano Zn substantially increased plant height, LAI, dry matter production, number of grains per cob, grain yield, stover yield and harvest index.

Hameed *et al.* (2021) reported that in cabbage, fertilization with nano-fertilizers, higher average of head circumferences, higher head height average, higher average of cabbage head weight and increased chlorophyll content of plant leaves were seen.

Ajithkumar *et al.*(2021) reported that in maize crop, among different combinatios of nanofertilizers with recommended dose of fertilizers the treatment

T11 (50% N, 100% PK,+ 2 sprays of IFFCO nano N (4 ml/L) mixed with IFFCO Sagarika (2ml/L) showed significant effect on the growth and yield parameters whereas, treatment T10 (50% N, 100% PK, + 2 sprays of IFFCO nano N (4ml/L) mixed with nano Zn (2 ml/L) and IFFCO nano Cu (2ml/L) was found superior with regard to management of Turcicum leaf blight disease with minimum of 18.20% severity.

Tiwari *et al.*(2021), reported that in potato quantity of urea being applied by the farmers to supply nitrogen to the crop can be successfully reduced to half. The yields obtained with 50% less nitrogen as compared to FFP and applying two sprays of nano nitrogen in standing crops gave yields higher than FFP in most of these trials.

Lahari *et al.* (2021) reported that in rice, 50% recommended dose of nitrogen + 50% nano nitrogen increased the plant height, number of tillers, grain yield and straw yield.

Vishekaii (2021) reported that the nano-nitrogen application improved oil quality attributes such as TPC and antioxidant capacity in olive. Based on the promising results, the application of nitrogen, particularly in the form of nano-nitrogen, should be included in orchard management approach that aims at producing high quality oils.

Anil Kumar *et al.* (2022) reported that in cereal and oilseed crops, the intervention of nanotechnology along with organic farming practice can help in minimizing the mass volume requirement of conventional chemical fertilizer while improving crop production.

Priyadarshana *et al.* (2022) reported that in Bermuda grass, plots treated with nano-urea showed improved growth response than the pots treated with granular urea. Treatments with 5ml/L of water nano-urea foliar spray recorded the highest values for almost all the morphological and physiological parameters.

Mohammad Saud *et al.* (2022) reported that in rice, application of Bio Organic Fertilizer in conjunction with chemical fertilizers and nano-urea spray reduced the N loss and boosted availability throughout the crop growth period by forming organic-mineral associations therefore increasing the yield and the foliar treatments resulted in more effective nitrogen absorption and assimilation in crop production.

Satadal Samata *et al.*(2022) reported that in finger millet application of 20kg N/ha + 2 sprays of IFFCO nano-urea @ 4ml/L could be economical and sustainable strategy to optimize the use of nitrogen in finger millet in southern Odisha.

Sumanta Samui *et al.* (2022) reported that in rabi maize, adoption of 100% RDN + foliar spray of nano-urea @ 4ml/L, twice at knee stage and tasseling stage was superior in influencing morphology, yield and yield attributes.

2.2 Nanoparticle delivery in the plant system

Kurepa *et al.* (2010) reported uptake and translocation of TiO_2-alizarin red-S complex in *Arabidopsis thaliana* seedling. They observed that mucilage released by root develops pectin hydrogel complex around the root found to be responsible for the entry of nanoparticle-dye complex.

Sun *et al.* (2014) carried out an experiment to study the mechanism of nanoparticle uptake and translocation fluorescently labelled monodispersed mesoporous silica nanoparticles were found to penetrate thr roots via symplastic and apoplastic pathways and translocated through xylem tissue to the aerial parts of the plant including the stem and leaves.

Abdel Aziz *et al.* (2016) conducted an experiment to investigate the delivery of chitosan nanoparticles loaded with nitrogen, phosphorus and potassium (NPK) for wheat plants foliar uptake. Chitosan-nanoparticles were easily applied to leaf surfaces and entered the stomata via gas uptake, avoiding direct interactions with soil systems. He found that nano particles were taken up and transported through phloem tissues which increased harvest index, crop index and mobilization index of wheat plants as compared to normal fertilized NPK. The lifecycle of the nano-fertilized wheat plants was shorter than normal-fertilized wheat plants with the ratio of 23.5% (130 days compared with 170 days for yield production from the date of sowing). He also found that the response of plants to nano fertilizers varies with the type of plant species, their growth stages and nature of nano materials.

2.3 Effect of nanofertilizers on yield and yield components

Melendi *et al.* 2008 reported that nanoscale devices are envisioned that would have the capability to detect and treat an infection, nutrient deficiency, or other problem, long before symptoms were evident at the macro-scale and nanoparticles as smart treatment delivery system.

Al Jabri (2009) conducted series of experiments to study the effect of zeolite as soil conditioner in the nitrogen (N) uptake, and dry rice grain yield in the greenhouse and the field condition. The zeolites in this research were zeolite with trade mark Zeolite Kap Kan (ZKK). The results show that the higher addition of ZKK zeolite, the higher N concentration was observed. The highest N concentration (2.96%) was obtained at dosage of 1 ton ZKK ha-1. The weight of dry rice grain yield in the greenhouse increased with the addition of Zeo Nano level 2 (Zeo-Nano was formulation of mixing of zeolite with straw of rice compost, urea, ammonium sulphate, rock phosphate, micronutrient Cu, Zn, and B in the pellet form). 6.52 ton ha^{-1} of dry rice grain has been reached by the zeolite addition of 2 ton ZKK ha^{-1}.

Kalpana Sastry *et al.* 2009 reported that in the management aspects, efforts are made to increase the efficiency of applied fertilizer with the help of nano clays and zeolites and restoration of soil fertility by releasing fixed nutrients. In the controlled environment agriculture and precision farming requirement of crops are diagnosed based on needs and required quantities are delivered in right time at right place with the help of nano biosensor and satellite system.

Cui *et al.* 2011 reported that Nano structured formulation through mechanisms such as targeted delivery or slow/controlled release mechanisms and conditional release, could release their active ingredients in response to environmental triggers and biological demands more precisely. The use of nano fertilizers causes an increase in nutrient use efficiency, reduces soil toxicity, minimizes the potential negative effects associated with over dosage and reduces the frequency of the application. Hence, nanotechnology has a high potential for achieving sustainable agriculture, especially in developing countries.

Ali *et al.* 2014 reported that Nano herbicides are being developed to address the problems in perennial weed management and exhausting weed seed bank.

Armin *et al.* (2014) conducted field experiment to study the effect of time and concentration of Nano-Fe foliar application on yield and yield components of wheat and observed that foliar application of Nano-Fe foliar spray at tillering + stem elongation and tillering had 9.17% and 5.19% more grain yield. Foliar application of Fe at 2%, 4% and 6% produced an increase of 12%, 22.09% and 19.07% grain yield over the control.

Bakhtiari *et al.* (2015) conducted field experiment on loamy sand soil to study the effect of Iron Nano particles concentration on wheat and revealed that among the different concentrations of iron Nano-oxide the highest spike weight (g), 1000-grain weight (g), biological yield (kg/ha), grain yield (kg/ha) and protein content (%) was recorded with the 0.04% concentration whereas the lowest spike weight (g), 1000-grain weight (g), biological yield (kg/ha), grain yield (kg/ha) and protein content (%) was recorded with Control.

Benzon *et al.* (2015) conducted experiment to determine the effects of nano fertilizer application on the yield, total phenolic content (TPC) and antioxidant activity of rice compared with conventional fertilizers. Results showed that agronomic parameters were significantly enhanced by nano fertilizer application as it promoted the growth, development, TPC, and antioxidant activity in rice, showing the potential to improve crop production and plant nutrition over conventional fertilizers.

Hafeez *et al.* (2015) conducted an experiment to determine the potential of copper nanoparticles for enhancing growth and yield of wheat and observed that addition of copper nanoparticles up to 0.4 ppm significantly increased the leaf area, chlorophyll content, plant fresh weight, dry weight and root weight over control. Further increase in the level of copper nanoparticles caused significant drop in value of the growth parameters except plant fresh weight that started decreasing at 0.8ppm. This might be due to more bioavailability, absorption and accumulation of nanoparticles leading to toxic effect. The best results i.e., umber of spikes/plant. 100-grain weight and grain yield per pot were achieved with the application of 30 ppm copper nanoparticles.

Hussain *et al.* (2015) conducted an experiment in the greenhouse conditions to investigate the effect of nano-fertilizer on mineral status of cotton plants grown under water stress. The treatments were as follows: a) Water stress treatments: Missing of irrigation at budding (D1) and flowering stages (D2) more than regular irrigation (R1) as control. b) Fertilizer treatments: 0.5 and 1.0 g nano phosphorus (nano-P) and distilled water as a control. Nano-fertilizer affects the macronutrient and micronutrient status under different irrigation treatments. Application of nano-P improved the nutrient uptake under stress conditions as well as regular irrigation. The interaction effect of nano-fertilizer and drought through some growth stages of cotton plants indicated the application of nano-P at 0.5 g promotes the nutrient uptake under D1, while 1.0g depicted the best nano-P fertilizer rate and enhanced the nutrient uptake under D2 condition.

Nethravathi *et al.* (2015) conducted a comparative study between the nanosized and granular fertilizers on the plant growth and development.

Chemically synthesised fertilizers in granule form were grinded to powder form. Fertilizers were available to crop plant effectively thereby increasing the uptake of fertilizers. These in turn reduces the loss of fertilizers by the supply of balanced and sufficient amount in nanosize. The growth rate of plant was found to be more when fertilizers were used in nano scale compared to the growth rate of plant with normal fertilizers in granular form.

Bahri *et al.* (2016) studied the effect of silver nanoparticles on different parameters such as seed germination and seedling biology of *Vigna radiata* (mung bean). Seeds of *Vigna radiata* were cultured under invitro conditions on MS nutrient medium (Murashige and Skoog's medium, strength 1/20) fortified with filter sterilized silver nanoparticles at concentrations of 10 ppm (parts per million), 20 ppm, 50 ppm and 100 ppm. MS medium without nanoparticles served as the control. Addition of silver nanoparticles at a concentration of 10 ppm enhanced percent seed germination in comparison to the basal medium. Shoots and roots of the seedlings raised attained maximum average length at this level of silver nanoparticles as compared to the control.

Davarpanah *et al.* 2016 conducted an experiment in pomegranate (*Punica granatum* cv. *ardestani*) to assess the effects of the foliar application of nano-fertilizers of zinc (Zn) and boron (B) on fruit yield and quality. A single foliar spray with relatively low amounts of B and Zn nano-fertilizers (34 mg B tree−1 and 636 mg Zn tree−1, respectively) led to increase in pomegranate fruit yield, and this was mainly due to increase in the number of fruits per tree. The effect was not as large with Zn as with B. Fertilization with the highest of the two doses led to significant improvements in fruit quality, total soluble salts, maturity index and pH and decrease in total acid in juice whereas physical fruit characteristics remains unaffected.

Hasaneen *et al.* 2016 studied the effect of foliar application of engineered Nano materials: carbon Nano tubes NPK and chitosan Nano particles NPK fertilizer on the growth of French bean. The results of the combined morphological and anatomical analysis indicate that after about 30 days from the date of planting, Nano materials either alone or in combination significantly enhanced plant growth and biomass compared to control.

(Manjunatha *et al.* 2016) and Subramanian *et al.* (2012) presented that nano-based smart delivery systems should go beyond the boundaries of foliar feeding and suggested that there is an abundance of scope of exploiting smart delivery systems in agriculture which facilitate enhanced use efficiency of inputs besides environmental protection. It is time that agricultural scientists should undertake research in the fascinating field of nano-based smart delivery systems so as to achieve the targeted delivery of inputs that enhance the crop productivity of crops with minimal use agri-inputs.

Manjunatha *et al.* (2016) studied the applications of nano fertilizers with the growing limitation in arable land and water resources and observed that the development of agriculture sector is only possible by increasing resources use efficiency with the minimum damage to agro ecology through effective use of modern technologies. Among these, nano technology has the potential to revolutionize agricultural systems, biomedicine, environmental engineering, safety and security, water resources, energy conversion, and numerous other areas.

Aghajani and Soleymani (2017) studied yield and yield components of pinto beans under drought stress (water deficit) conditions. The treatments including: 1) control (0), 2) Nano Biologic, 3) Nano Biologic with 75 Kg pure N (urea, surface broadcast), 4) 150 Kg pure N (urea), 5) 75 Kg pure N with nano Zn, Fe, Mn (foliarly applied), and 6) 75 Kg pure N with Zn, Fe, Mn (foliarly applied) were used as the subplots. Yield and yield components of pinto beans were determined. The results indicated that although drought adversely affected bean growth and yield production, nano fertilization types including 3 and 5 resulted in the highest rate of yield and yield components under water sufficient and deficient conditions.

Davarpanah *et al.* (2017) conducted experiment to compare the effects of foliar fertilization with urea and nitrogen (N) fertilizer containing nanoparticles (nN) on the characteristics of pomegranate fruits cv. Ardestani during two consecutive years. Two foliar applications of nN (0.25 and 0.50 g N L-1 respectively) and urea (4.6 and 9.2 g N L-1 respectively) were applied at full bloom and one month later, and trees not treated with any N fertilizer were used as a control. Results show that foliar N fertilization increased fruit yield (by 17% to 44%) and number of fruits per tree (by 15% to 38%). The highest fruit yields (17.8 and 21.9 kg per tree) and number of fruits per tree (62.8 and 70.1 per tree) were obtained with the treatment nN2 (1.8 kg N ha-1), whereas the lowest fruit yields (12.4 and 16.2 kg per tree) and number of fruits per tree (45.5 and 55.3 per tree) were recorded in the control trees. The treatments U1 and nN2 increased fruit length (the latter only in the second season), whereas the treatment U1 increased average fruit weight (10% to 11%) in both seasons. The treatment nN2 increased aril juice and total soluble solids (TSS) in both seasons and titratable acidity (TA) only in the first season, whereas the treatment U1 increased TSS in both seasons and aril juice and TA in the second season. Pomegranate fruit yield was improved with two applications (at full bloom and one month later) of nN fertilizer at a rate of 1.8 kg N ha^{-1} and with two applications of urea at a rate of 16.3 kg N ha^{-1}. Furthermore, fruit quality was improved more with the nN fertilizer at a rate of 1.8 kg N ha^{-1} than with two applications of urea at a rate of 16.3 kg N ha^{-1}.

Jyothi and Hebsur (2017) while studying the effect of nano-fertilizers on the growth and yield of selected cereals observed that the full recommended rate of conventional dose of nano fertilizers (FRR-CF+FRR-NF) enhanced the plant height, chlorophyll content, number of reproductive tillers, panicles and spikelet's in rice.

Jyothi and Hebsur (2017) reported that excessive use of fertilizers, pesticides and insecticides also caused several health issues in population. Despite these problems there is also challenge to feed the growing population of country. Therefore in future, there is need to produce nutritive agricultural produce rich in protein and other essential nutrient required to the human and animal consumption that is why emphasis should be laid on production of high quality food with required quantity of nutrients and proteins. For solving these problems in crop production Nano-fertilizers, pesticides may be the effective tools in agriculture for better pest and nutrient management.

Saedpanah *et al.* (2017) conducted an experiment on maize to study agronomic traits with treatments of nano fertilizers as foliar spray including iron chelate, zinc, potassium and NPK fertilizers and control. The subplots consisted of the soil application of zeolite including 1000 g m^2, 500 g m^2 and control. Results showed that the plant height improved with fertilizer applications under no application of zeolite. Plant height increased by 12% with Fe, 14% with K, 14% with zinc and 16% with macro elements (NPK) in non-zeolite application treatment. The total biomass was increased with iron and NPK fertilizers applications under no application of zeolite. Total biomass increased by 30% for iron, 31% for potassium, 10% for zinc, 40% for NPK and 15% for control when zeolite application increased from 0 to 1000 g m^2. The highest leaf (5556 kg ha^{-1}) and stem biomass (3851 kg ha^{-1}) were obtained in NPK treatment. Leaf and stem biomass was increased as zeolite levels increase. The highest leaf (5644 kg ha-1) and stem biomass (3504 kg ha-1) were obtained in 1000 g m^2 treatment.

Singh *et al.* (2017) reported that nano fertilizers are the important tools in agriculture to improve crop growth, yield and quality parameters with increase nutrient use efficiency, reducing wastage of fertilizers and cost of cultivation. Nano-fertilizers are very effective for precise nutrient management in precision agriculture with matching crop growth stage for nutrient and may provide nutrients throughout the crop growth period. Nano-fertilizers increase crop growth up to optimum concentrations. Nano-fertilizers provide more surface area for different metabolic reactions in the plant which increase rate of photosynthesis and produce more dry matter and yield of the crop. It also prevents the plant from different biotic and abiotic stresses.

Chapter -3

MATERIALS AND METHODS

The present investigation entitled **"Impact of Nano-urea on Morpho-physiological, Biochemical and Yield Parameters of Kale (*Brassica oleracea* var. *acephala*)"** was a pot experiment carried out in the laboratory of Division of Basic Sciences & Humanities/ Division of Vegetable Sciences, Sher–e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar, J&K during the year 2021-2022. The details of materials used and techniques employed during the course of investigation are presented in this chapter.

3.1 Seed source

The seeds of Kale (GM Dari and Khanyari) were obtained from Seed Production Unit of Division of Vegetable Science, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar.

3.2 Temperature and Relative humidity

The temperature and relative humidity were maintained in the laboratory during the entire experiment. The temperature was 25±2°C and the RH was maintained at 80-90%. An additional work was done in field also. For that the mean weekly meterological data recorded at Faculty of Horticulture, SKUAST-K during the cropping period is given in Appendix-1 and Fig.1

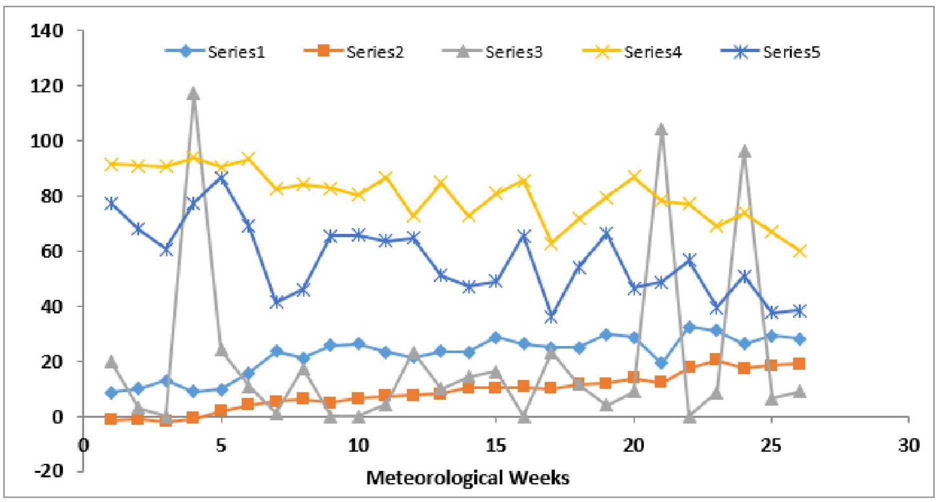


Fig. 1: Meteorological observations during the cropping period

3.3 Soil characteristics

The physico-chemical properties of the soil used for the study are given below in Table-1

Table-1: Physico-chemical properties of the soil

Soil characteristics	Value
pH	6.40
E.C(dSm^{-1})	0.45
N(Kg ha^{-1})	200.01
P(Kg ha^{-1})	19.01
K(Kg ha^{-1})	146.60

3.4 General description

3.4.1 Soil preparation and pot filling

Soil was collected from the experimental field of Division of Vegetable Science, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar. The soil was mixed with farm yard manure in a ratio of 2:1. The pots of uniform size (27×24.5 cm) and weight (0.370 Kg) were filled with 8.0 Kg of soil. Little amount of sand was mixed thoroughly in each pot to improve aeration.

3.4.2 Fertilizer application

The recommended dose of phosphorus (100 Kg/ha or 1.73g/pot) and potassium (60 Kg/ha or 1.46g/pot) were applied through SSP and MOP in each treatment. The recommended dose of nitrogen (120 Kg/ha or 2.4g/pot) was provided to the first treatment (T_1) through conventional urea, to the third treatment (T_3) through urea spray and to the other treatments through the different concentrations of nano-urea foliar spray (T_4-T_8), which were given at 15 days interval starting at 15 DAS upto 45 DAS. No nitrogen in any form was given to T_2 (Control).

3.4.3 Sowing and irrigation

Seeds of each variety were sown in pots and in each pot 10 seeds were sown. Pots were watered alternately till seedling establishment and after that at an interval of 2 -3 days till the establishment of plant.

3.4.4 Cultural practices

Cultural practices and plant protection measures like weeding, hoeing, irrigation and insecticide applications were carried out as and when required.

3.5 Technical programme

Target crop	:	Kale (*Brassica oleracea* var. *acephala*)
Location	:	Division of Basic Sciences and Humanities/Division of Vegetable Sciences, SKUAST-K Shalimar
Varieties	:	1. GM Dari 2. Khanyari
No. of treatments	:	08
		T_1: urea (recommended soil application)
		T_2: control (no urea application)
		T_3: urea spray (2000 ppm)
		T_4: nano-urea spray (2000 ppm)
		T_5: nano-urea spray (1000 ppm)
		T_6: nano-urea spray (500 ppm)
		T_7: nano-urea spray (250 ppm)
		T_8: nano-urea spray (125 ppm)
Replications/Treatment	:	03
Design of experiment	:	CRD(factorial)

The above mentioned treatments (T3-T8) were applied at 15, 30 and 45 days after sowing.

3.6 Treatment details

The treatments used for the current study were as follows

Table 2: Treatments used in the experiment

Notation	Treatment
T_1	urea (recommended soil application)
T_2	control (no urea application)
T_3	urea spray (2000 ppm)
T_4	nano-urea spray (2000 ppm)
T_5	nano-urea spray (1000 ppm)
T_6	nano-urea spray (500 ppm)
T_7	nano-urea spray (250 ppm)
T_8	nano-urea spray (125 ppm)

3.7 Observations recorded

Observations on below mentioned attributes as per the approved technical programme were recorded following the standard procedures:

Plant growth parameters:

- Plant height (cm)
- No of leaves per plant
- Leaf area per plant (cm^2)
- Fresh weight of leaves (g/plant)
- Dry weight of leaves (g/plant)

Physiological parameters

- Net photosynthetic rate (µmole m^{-2} sec^{-1})

- Stomatal conductance (mmole m^{-2} sec^{-1})
- Transpiration rate (µmole m^{-2} sec^{-1})
- Leaf chlorophyll a content (mg g^{-1} FW)
- Leaf chlorophyll b content (mg g^{-1} FW)
- Total leaf chlorophyll content (mg g^{-1} FW)
- Leaf carotenoid content (mg g^{-1} FW)
- Net assimilation rate (g m^{-2} day^{-1})
- Relative growth rate (g g^{-1} $day^{-1)}$

Biochemical parameters

- Total Phenols (mg GAE g^{-1})
- Total carbohydrates (g/100g)
- Leaf N content (%)
- Leaf P content (%)
- Leaf K content (%)
- Leaf Ca content (%)
- Leaf Nitrate content (mg/100g)

Molecular parameters

- Protein analysis of leaf samples (SDS page)

Quality parameters

- Vitamin C (mg/100g)
- Total Antioxidant activity (mg GAE g^{-1})
- Protein content (g/100g)
- L*a*b*(colour)

Yield parameters

- Yield per plant (g)
- Yield per hectare (t $ha^{-1)}$

Plate 1: Cultural practices

3.8 Details of methodologies followed

3.8.1 Plant height (cm)

Plant height of each replication was measured in centimeters as the height from ground level to the tip of plant in cm with the help of a meter scale and their average worked out in centimeters.

3.8.2 Number of leaves per plant

The number of leaves from each plant was counted and the average from three replications was worked out.

3.8.3 Leaf area per plant (cm^2)

Leaf area of the individual plant was estimated with the help of following equation available in the literature (Khan *et al..,* 2016) and expressed in terms of cm^2 $plant^1$.

$$LA = 0.347(L \times W)-10.7$$

Where, L=Length of leaf and W=width of leaf

3.8.4 Fresh weight and dry weight of leaves (g/plant)

All the leaves of a single plant in a pot were plucked and the average fresh weight per plant was calculated for every treatment. The dry weight of the same plucked leaf samples was taken after drying the leaves at 40°C for 48 hours in oven. The dried leaf samples were weighed using an electronic balance and expressed as grams.

3.8.5 Net photosynthetic rate (µmole m^{-2} sec^{-1}), Transpiration rate (µmole m^{-2} sec^{-1}) and Stomatal conductance (mmole m^{-2} sec^{-1})

Net photosynthetic rate, transpiration rate and stomatal conductance of Kale leaves were measured by using Infrared Gas Analyser (IRGA-TPS2). It is the completely self-contained unit for measuring the CO_2 assimilation (photosynthesis), transpiration (water loss by evaporation of leaves of plant) as well as stomatal conductance. It operates on the "open system principle". The leaf was placed in a sealed enclosure with a window for illumination referred to as leaf cuvette. A measured flow of air was passed through the cuvette. The CO_2/H_2O

concentration of the air entering and leaving was measured. To measure the concentration of CO_2/H_2O TPS-2 uses a single CO_2 and water sensor and alternatively switches the reference and analysis air. From the rate flow of air and the change in concentration of CO_2/H_2O, the assimilation and transpiration rate as well as stomatal conductance were calculated.

3.8.6 Chlorophyll content (a, b and total)

The contents of chlorophyll-a, chlorophyll-b and total chlorophyll were estimated according to the method given by Hicox and Isrealstam (1979) using dimethyl sulphoxide (DMSO) as extractant. Fresh leaf samples were chopped into small pieces excluding mid rib. 100 mg of chopped leaf samples were put into test tubes already filled with 10 ml of DMSO. The test tubes were then covered by aluminium foil. After keeping in the oven for about four hours at 45°C, the supernatant was carefully transferred into a volumetric flask and volume was made to 20 ml. The absorbance of extract was measured at 645 and 663 nm using double beam UV-VIS spectrophotometer (UV-570488) against DMSO as blank. The amount of chlorophyll pigments were calculated using the formulae given by Arnon (1949). The amount of chlorophylls were expressed as mg. g^{-1} fresh weight of leaf.

$$\text{Chlorophyll } a = [(12.7 \times A_{663}) - (2.69 \times A_{645})] \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll } b = [(22.9 \times A_{645}) - (4.68 \times A_{663})] \times \frac{V}{1000 \times W}$$

$$\text{Total Chlorophyll } a+b = [(8.02 \times A_{663}) + (20.2 \times A_{645}) \times \frac{V}{1000 \times W}$$

Where,

A = Absorbance at specific wave length

V = Final volume of DMSO in ml and

W = Fresh weight of tissue extracted in grams (g)

3.8.7 Leaf carotenoid content (mg g^{-1} FW)

Extraction of leaf carotenoid content was done by the method of Hiscox and Israelstam (1979) using dimethyl sulfoxide (DMSO) as extractant. Leaf samples were prepared as in case of chlorophyll. 100 mg of leaf samples were kept in 10 ml of dimethyl sulfoxide (DMSO) in test tubes. The test tubes were then covered by aluminium foil and kept in hot air oven at 45°C, the supernatant was carefully transferred into a volumetric flask and volume was made to 20 ml. The absorbance was measured on spectrophotometer at 480 nm for determination of total carotenoid as per the formula given below (Wellburn 1994):

$$\text{Total carotenoids } (\mu g\ ml^{-1}) = \frac{1000 \times A_{480} - (2.14 \times Ca) - (70.16 \times Cb)}{220}$$

Where,

Ca = Chlorophyll *a* ($\mu g\ ml^{-1}$) = $[(12.19 \times A_{665}) - (3.45 \times A_{649})]$

Cb = Chlorophyll *b* ($\mu g\ ml^{-1}$) = $[(21.99 \times A_{649}) - (4.68 \times A_{665})]$

3.8.8 Net assimilation rate (g m^{-2} day^{-1})

NAR is defined as the increase of dry matter per unit leaf area per unit time (g/m^{-2}/day) (Gul *et al..,* 2013).

$$NAR = [(W_2-W_1)/ (T2-T1)]\times[(\text{In } L2 - \text{In } L1)/(L2-L1)]$$

Where,

W1 and W2 are dry weight of plant at time T1 and T2

L1 and L2 are leaf area at T1 and T2.

3.8.9 Relative growth rate (g g^{-1} $day^{-1)}$

Relative growth rate (RGR) is an improved technique to measure the growth rate of plants as it takes into consideration the initial dry weight of the plant and expressed in terms of g $g^{-1}day^{-1}$. It is similar to compound intrest wherein the increment in any interval adds to the capital for subsequent growth. It was measured following the method of Fisher (1921).

$$RGR(g\,g^{-1}day^{-1}) = \frac{2.302\ (\log_e W_2 - \log_e W_1)}{T_2 - T_1}$$

Where,

W_1 = plant dry weight

W_2 = plant dry weight (g) at time t_2

Loge = natural logarithms (Logarithms to the base of 2.3026)

3.8.10 Total phenols (mg GAE g^{-1})

Preparation of standard

The total phenolic content in the plant extracts was determined by using Folin-Ciocalteu colometric method based on oxidation-reduction reaction (Waterhouse 2002). Various concentrations of gallic acid solutions in methanol (10, 25, 50 and 75µg/ml) were prepared. In a 20 ml test tube, 1 ml gallic acid of each concentration was added and to that 5 ml of Folin-Ciocalteu reagent (10%) and 4 ml of 7% Na_2CO_3 were added to get a total volume of 10 ml. The blue colored mixture was shaken well and was incubated for 30 minutes at 40°C in a water bath. Then the absorbance was measured at 760 nm against blank. All the experiments were carried out in triplicate. The average absorbance values obtained at different concentrations of gallic acid were used to plot calibration curve.

Preparation of sample

Various concentrations of extracts (25, 50, 100 and 200 µg/ml) were prepared. Following the procedure described for standard, absorbance for each concentration of extract was recorded. Total phenolic content of the extracts was expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g).

The total phenolic contents in all samples was calculated using the formula:

$C= cV/m$

Where, C= total phenolic content mg GAE/g dry extract

c = concentration of gallic acid obtained from calibration curve in mg/mL

V= volume of extract in ml, m= mass of extract in gram.

3.8.11 Total carbohydrates (g/100g)

The total carbohydrates were estimated by Phenol-sulphuric acid method. Simple sugars, oligosaccharides, polysaccharides and their derivatives give green colour when treated with phenol and conc. H_2SO_4. The reaction is sensitive and the colour is stable.

Principle

A hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This forms green coloured product with phenol and has absorption maximum at 490 nm.

Reagents

1. 5% Phenol: Dissolve 50 g of redistilled (reagent grade) phenol in water and dilute to one litre.
2. 96% Sulphuric acid (reagent grade).
3. Standard glucose (stock): 100 mg in 100 ml of water.
4. Working standard: 10 ml of stock diluted to 100 ml with distilled water.

Procedure

1. Followed the steps (1 to 4) of anthrone method for sample preparation.
2. Pipetted out 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard into a series of test tubes.
3. Pipetted out 0.1 and 0.2 ml of the sample solution in two seprate test tubes. Made the volume in each tube to 1 ml with water.
4. Set a blank with 1 ml of water.
5. Added 1 ml of phenol solution to each tube.

6. Added 5 ml of 96% sulphuric acid to each tube and shook well.

7. After 10 min, shook the contents in the tubes and placed in a water bath at 25-30°C for 20 min.

8. Read the colour at 490nm.

9. Calculated the amount of total carbohydrates present in the sample solution using the standard graph.

Calculation

Absorbance corresponds to 0.1 ml of the test tube = *x* mg of glucose

$$10 \text{ ml contains} = \frac{x \times 10 \text{ mg of glucose}}{0.1} = \text{\% of total carbohydrate present}$$

3.8.12 Leaf Nitrogen (%)

Total nitrogen content of the samples was estimated by modified Kjeldahl method (Jakson, 1973).

Principle

The sample is digested with sulphuric-salicylic acid. Orgaic and nitrate nitrogen is converted to ammonium sulphate and the ammonia gas is distilled into boric acid and titrated with standard sulphuric acid.

Reagents

Sulphuric salicylic acid, sodium thiosulphate, sulphate mixture, sodium hydroxide (40 percent), 0.005 N standard sulphuric acid and boric acid indicator.

Procedure

In this method, in 100ml Kjeldahl's flask, 0.5g of dried sample was taken in 20ml of sulphuric salicylic acid mixture (1g of salicylic acid and 30ml of conc.H_2SO_4) were added and swirled so that dry sample comes in contact with reagents. After keeping overnight, 5g of sodium thiosulphate was added and flask was heated gently for about 5 min. After cooling the sample, 10g of sulphate mixture (20 parts of K_2SO_4 and 1 part of catalyst mixture) were also added and then the sample was put in Kjeldahl's apparatus for digestion at full heat. The digestion was done for 1 hour. Glass beads were added to avoid bumping during the digestion. After completion of digestion, digest was cooled and diluted to 100 ml of sodium hydroxide (NaOH).

Ten milliliters of 4 percent boric acid solution containing bromocresol green and methyl red indicator was taken in a conical flask and flask was placed in such a way that condenser outlet of distillation apparatus was diped into the boric acid solution. Ten milliliters of aliquot (digest) was taken and transferred to distillation flask of micro-Kjeldhal distillation apparatus. After adding the aliquot, the funnel of the apparatus was washed with 2-3 ml of distilled water and 10 ml of 40 percent NaOH solution was added. About 5 ml aliquot was distilled to boric acid containing flask. After completion of distillation, the boric acid was titrated against 0.005 N H_2SO_4. Blank was run in the same way as that of sample. The total nitrogen was calculated by the following formula:

$$\text{N (per-cent)} = \frac{0.00007 \times \text{titration value} \times 100 \times 100}{\text{Aliquot taken (ml)} \times \text{wt. of sample (g)}}$$

3.8.13 Leaf phosphorus (%)

Principle

Total phosphorus content was estimated by Vanado-molybedate-phosphoric method after digestion in diacid mixture (HNO_3: $HClO_4$ in the ratio of 9:4). During digestion phosphorus is converted to orthophosphates. These orthophosphates react with molybedate and vanadate and give yellow coloured unreduced vanado-molybdo-phosphoric complex in acid solution (Jackson, 1973).

Reagents used

Ammonium molybedate, ammonium vandate, nitric acid (NHO_3), perchloric acid ($HClO_4$) and phosphate standard solution.

Procedure

Samples were digested in a di-acid mixture of nitric acid and perchloric acid (HNO_3: $HClO_4$, 9:4). Five milliliter of extract was transferred to 50 ml volumetric flask, 10 ml of vanadomolybdate reagent was added and the volume was made upto mark with deionized or distilled water. The absorbance of solution was measured after 30 minutes at 420 nm in spectrophotometer using blue filter.

Standard solutions of 0, 1, 2, 3, 4 and 5 ml of phosphorus were transferred to 50 ml volumetric flask to get 0,1, 2, 3, 4 and 5 mg Kg^{-1} of phosphorus respectively. The absorbance of solution was read after 30 min at 420 nm in spectrophotometer. The absorbance was then plotted against concentration and the total phosphorus was calculated from the following formula:

$$\text{P (per-cent)} = \frac{C}{\text{Wt. of sample}} \times \frac{100}{\text{Aliquot taken}} \times \frac{\text{Volume of digest}}{10000}$$

Where,

C = Concentration obtained from standard curve (ppm)

3.8.14 Leaf Potassium (%)

Total potassium content was estimated with the help of Flame Photometer.

Principle

The determination is based on measurement of the spectral line intensities of potassium atoms excited when passed through a flame. When a solution of salt is sprayed into the flame, it excites the atoms to higher energy levels, they emit radiations. The intensity of these emitted radiations is proportional to the concentration of the particular element present in the sample.

Chemicals used

Standard potassium chloride solution, nitric acid (NHO_3) and perchloric acid ($HClO_4$).

Procedure

The samples were digested in diacid (HNO_3: $HClO_4$, 2:1)

A series of standard solution of potassium (0, 5, 10 mg Kg^{-1}) were prepared. The flame photometer was set with solution of highest concentration (i.e., 10 mg Kg^{-1}). Readings of other standards were then taken and curve was plotted.

The digest was diluted to suitable concentration range so that the final concentration lied between 0 to 5 mg Kg^{-1}. The samples were then read in flame photometer at 548 nm wavelength using filter for potassium. The total potassium was calculated from the following formula:

$$K(\text{per-cent}) = \frac{R \times 5 \times}{100} \times \frac{100}{\text{Sample taken (g)}} \times \frac{100}{1000000}$$

Where,

5 mg Kg^{-1} K = 100 reading

R = Flame photometer reading

3.8.15 Leaf Calcium (%)

Calcium concentration of kale leaf was estimated by digesting the fully dried powder (1 g) with di- acid (perchloric acid and nitric acid in the ratio of 9:4). The sample was then heated and volume made upto 100 ml. The sample was filtered through Whatman No. 1 filter paper and was collected in volumetric flask. The calcium standard was prepared and reading were recorded with the help of atomic absorption spectrophotometer. The value of calcium was expressed in per-cent (Interference in the calcium estimation is lowered by adding 2% strontium while making standard).

3.8.16 Leaf Nitrate content (mg/100g)

The salicylic acid method is the most reliable and stable method to evaluate the nitrate content in plant tissues. In this method, nitrosalicylic acid is formed by the reaction of nitrate and salicylic acid under highly acidic conditions. The complex is yellow under basic (pH>12) condition with maximal absorption at 410 nm . The absorbance is directly proportional to nitrate content. Therefore the nitrate content in the tissues can be calculated based on their absorbances. This method is suitable for determination of nitrate concentration in plants (Lufei Zhao and Yong Wang, 2017).

Procedure

A. Standard curve

1. To make the standard curve, 1 ml, 2 ml, 3 ml, 4 ml, 6 ml, 8 ml, 10 ml, and 12 ml nitrate standard solution (500 mg/L) was transferred to eight 50 ml flasks respectively, and deionized water was added to each solution to bring the total volume to 50 ml. The concentration of the series of standard solution should be 10, 20, 30, 40, 60, 80, 100, and 120 mg/L, respectively. And the molarity of 10, 20, 30, 40, 60, 80, 100, and 120 mg/L KNO_3 is 0.0007, 0.0014, 0.0021, 0.0029, 0.0043, 0.0057, 0.0071, 0.0086 mol/L, respectively.
2. Transfered 0.1 ml of each standard solution into a 12-ml tube, respectively. Used 0.1 ml deionized water as a control.
3. Added 0.4 ml salicylic acid-sulphuric acid into each tube and mixed well, and then incubated all reactions at room temperature for 20 min.
4. Added 9.5 ml of 8% (w/v) NaOH solution into each tube, cooled down the tubes (heat is generated due to the reaction) to room temperature (about 20-30 min), and measured the OD-410 value with the control for reference.
5. Plotted the standard curve with the nitrate concentration as the horizontal axis and the absorbance as the vertical axis. Then, the regression equation can be obtained based on the standard curve. The detailed methods are as follows:
   a) Open an Excel, enter the OD-410 values in column A and the nitrate concentrations of the standard solutions in column B. Select the cells containing values, and then insert a scatter plot.
   b) Select any data point in the plot, right click, select to add a trend line, choose the linear and display equation, then standard curve and the regression equation are obtained.

B. Nitrate assay

1. The seedlings, shoots, and roots are collected separately for nitrate content determination.
2. Froze each weighed sample ($\leq$ 0.1 g, for example about 20-25 7-day-old seedlings in a 1.5-ml tube by liquid nitrogen, and grind each sample into powder with the frequency of 30/sec for 1 min using a RETCH MM400.
3. Added 1 ml deionized water into the tubes and boil at 100 °C for 20 min (at least).
4. Centrifuged the samples at 15,871 x g for 10 min, and transfer 0.1 ml supernatant into a new 12ml tube. Used 0.1 ml deionized water as a control.
5. Added 0.4 ml salicylic acid-sulphuric acid into each tube, mixed the sample well, and then incubated the reactions for 20 min at room temperature.
6. Added 9.5 ml of 8% (w/v) NaOH solution into each tube and cooled down the tubes to room temperature (about 20-30 min). Measured the OD410 value of each sample with the control for reference.
7. According to the OD-410 value obtained in the above step, calculated the nitrate concentration (C) with the regression equation, C (µg/ml) = 140.86 x OD410 - 1.1831 obtained in the procedure A
8. Calculated the nitrate content using the following equation:

   $Y = CV/W$

Where,

Y: nitrate content (µg/g),

C: nitrate concentration calculated with OD-410 into regression equation as step B7 (µg/ml),

V: the total volume of extracted sample (ml),

W: weight of sample (g).

Plate 2: Estimation of leaf nitrate

3.8.17 Molecular parameter

Protein analysis of leaf samples (SDS page)

Protein extraction

SDS-PAGE was done by the method given by Singh and Krishnan (2013). In this method, one gram leaf tissue of each treatment was thoroughly grounded with mortor and pestle in liquid nitrogen to obtain fine powder. The powder was resuspended in 25 ml chilled acetone containing 10% TCA and 0.07% β-mercaptoethanol for reduction. The mixture was incubated at 20°C for 3 hours and centrifuged at 12000 rpm for 20 min at 4°C. The supernatant was decanted and 25 ml of 80% acetone with 0.07% mercaptoethanol and 2mM EDTA added. The mixture was vortexed vigorously and again centrifuged at 12000 rpm at 4°C for 20 min. The supernatant was decanted and above steps again followed until pellet obtained was completely free from chlorophyll. The pellet was washed with 100% acetone along with 0.07% β-mercaptoethanol and 2mM EDTA, centrifuged at 12000 rpm for 20 min at 4°C and supernatant decanted. Finally 2 ml of extraction buffer (containing of Tris-HCl (1M pH 8.8)-12 ml, EDTA (0.25M)-1.6ml, SDS (10%)- 40 ml, glycerol (50%)- 20 ml, and remaining distilled water in total 100 ml mixture) was added to pellet. The contents were mixed well and centrifuged at 12000 rpm for 20 min at 4°C. The supernatant containing total protein fractions (protein extract) were transferred to clean Eppendorf tubes and stored at -20°C for electrophoretic analysis.

Composition of 10% separating gel

Components	Volume
1.5 M Tris-HCl (pH 6.8)	7.5 ml
Water	12.3 ml
Acrylamide: Bisacrylamide	9.9 ml
10% SDS	0.15 ml
Ammonium per sulphate (10%)	70 µl
TEMED	70 µl

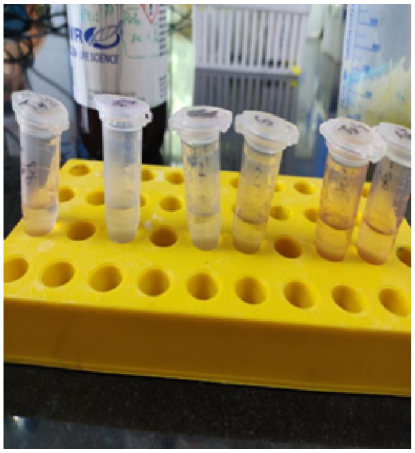
Composition of 4% stacking gel

Components	Volume
0.6 M Tris-HCl (pH 6.8)	1.25 ml
Stock-acrylamide	0.67 ml
10% SDS	0.025 ml
APS	0.025 ml
TEMED	0.005 ml
Water	3.075 ml

After, the gel got set, the comb was carefully removed from the stacking gel and the wells rinsed with buffer. The gel cassette was then reassembled in the electrophoretic tank and the reservoirs filled with electrophoresis buffer. Before loading of samples in wells, in a clean Eppendorf tube, 40µg of crude protein from lysate + 4µl of (0.4%) mercaptoethanol, 6µ of (0.4%) bromophenol blue, 2µl glycerol were mixed well. The tubes were then sealed and samples were boiled at 100°C for 5 minutes and 20µl of each sample were loaded into the appropriate well. The apparatus was connected to the power pack. The electrophoresis was continued till bromo-phenol blue reached to the bottom of the gel. The gel was removed from the cassette and then placed in staining solution. The staining step was followed by destaining process. The unstained protein ladder used in this study was from Biorad of low Range SDS-PAGE (175-22 kD).

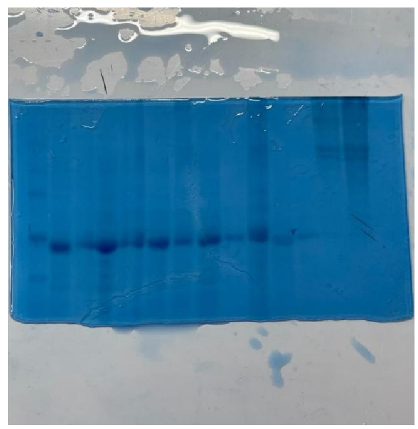
3.8.18 Vitamin C (Ascorbic acid content) (mg/100g) (AOAC, 1970)

Ascorbic acid was determined by titrating a known weight of sample with 2,6-dichlorophenol indophenol dye using metaphosphoric a stabilising agent.



Protein extraction

Eppendorf Tubes containing extracted proteins



Addition of extraction buffer to the pellets

Stained Gel

Plate 3: Protein profiling of leaf samples by SDS - PAGE

Known weight of the sample (100g) was ground in a pestle and mortar with stabilizing medium (1:5). Homogenate was centrifuged. Supernatant was taken and volume was made upto 50 ml with stabilizing medium and filtered. 10 ml of aliquot was titrated against 2,6-dichlorophenol indophenols dye. The dye factor was calculated by titrating standard ascorbic acid solution against dye and ascorbic acid content of the sample was expressed as:

$$\text{Ascorbic acid (mg } 100\text{g}^{-1}) = \frac{\text{Titre value} \times \text{dye factor} \times \text{volume made up}}{\text{Total weight of the sample} \times \text{aliquot of sample}} \times 100$$

3.8.19 Total antioxidant activity (mg GAE g^{-1})

The total antioxidant capacity of the plant extracts was measured by the method described by Prieto *et al.* (1999). The assay is based on the reduction of Mo(V1) to Mo(V) by the extract and subsequent formation of green phosphate/ Mo(V) complex at acid pH.

Reagents

Total antioxidant capacity (TAC) reagent consists of 7.45 ml of sulphuric acid (0.6 mM) solution, 0.9942g of sodium sulphate (28 mM) solution and 1.2359 g of ammonium molybdate (4 mM) solution mixed together in 250 ml distilled water.

Method

A leaf sample of 0.2 g was mixed with 1.5 ml of ethanol (80%) and macerated in mortor in dark. After centrifugation (14000 rpm × 15 minutes), an aliquot (0.1 ml) of extract was combined to 1 ml of reagent solution (0.6 mM sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated in a thermal block at 95°C for 90 minutes. After, the mixture had cooled to room temperature, this was diluted in distilled water (1:9) and the absorbance of each solution was measured at 695 nm against a blank. Blank was maintained with distilled water replacing the TAC reagent. The antioxidant capacity was expressed as gallic acid equivalent per gram dry weight (mg GAE/g DW). The calibration curve range was 0-100 µg/ml.

3.8.20 Protein content (g/100g) (Crude protein content determined on dry weight basis) (AOAC, 1965)

Crude protein was determined by Micro-Kjeldahl's method. A known weight of dry sample (100g) was take in a digestion flask and to it 1.9±0.1 mg potassium sulphate, 80±10 mg mercuric acid and 2 ml of sulphuric acid was added. The sample was digested after adding the boiling chips till the solution became colourless. The digest was cooled and diluted with a small quantity of double distilled ammonia free water and then final volume was made to 50 ml with distilled water. A 20 ml aliquot was then transferred to the distillation apparatus and another 100 ml conical flask containing 20 ml Boric acid and a few drops of mixed indicator was placed on the other end of Kjeldahl's apparatus with the tip of condenser dipping below the surface of the solution. 10 ml of sodium hydroxiode-sodium thiosulphate solution was added to the test solution in the apparatus. Distillation was started and ammonia was collected on boric acid. About 10 ml of distillate was collected, and it was titrated against standard sulphuric acid until the appearance of end point i.e, the violet color, the reagent was run blank with an equal volume of distilled water and titration volume was subtracted from that of sample titer volume and the percent protein was calculated by the following formula:

$$\%N = \frac{\text{(Sample titer (ml)-Blank titer)} \times 0.014 \times \text{Vol. of digest} \times \text{Normality of acid}}{\text{Aliquot taken (ml)} \times \text{Weight of sample (g)}} \times 100$$

% protein = %N×6.25

3.8.21 L*a*b*(colour)

As per the procedure given by Lamberts *et al.* (2006), a Hunter lab colorimeter (Model CM-508d Minolta Co., Japan) was used to measure the color of leaves using the L*-lightness vs. dark (0-50 = dark, 51-100 = light), a*-redness vs. greenness (positive value = red; negative value = green), and b*- yellowness vs. blueness (positive value = yellow; negative value = blue). Following relationships were used to compute chroma (C*) and hue angle (Hunt, 1991),

Chroma (C*) $=(a^2+ b^2)^{½}$

Hue angle(°) = 180 +arctan(b/a)

3.8.22 Yield plant^{-1} (g)

Leaf yield plant^{-1} was obtained by multiplying average leaf weight with average number of leaves for each treatment.

3.8.23 Yield hectare^{-1} (t)

After calculating leaf yield plant^{-1} by multiplying average leaf weight with average number of leaves for each treatment, then it is converted into plot and hectare basis.

3.9 Statistical analysis

Experimental data was subjected to the statistical analysis following procedures as described by Gomez and Gomez (1984). Level of significance used for F and t– tests were $p < 0.05$ from the table given by Fisher (1970) and the data collected was subjected to statistical analysis using MS Excel 2010 data analysis tool pack.

Chapter -4

EXPERIMENTAL FINDINGS

The results pertaining to the present investigation entitled **"Impact of Nano-urea on Morpho-physiological, Biochemical and Yield Parameters of Kale (*Brassica oleracea* var. *acephala*)"** are presented in this chapter.

4.1 Plant growth parameters

4.1.1 Effect of nano-urea on plant height (cm)

The data on plant height in both the varieties of Kale (Khanyari and GM Dari) is presented in Table 4.1. The data shows that the plant height is markedly influenced by foliar spray of nano-urea. The effect of different concentrations of nano-urea is statistically significant over control (i.e, no urea application). There is a significant difference in plant height due to the application of nano-urea treatments of varied concentrations. However, maximum plant height (88.50cm in Khanyari and 48.20cm in GM Dari) is observed for the treatment T_8 where the concentration of nano-urea used is 125 ppm which is followed by T_7 treatment (75.30 cm in Khanyari and 45.20 cm in G M Dari) where the concentration of nano-urea used is 250 ppm . The lowest plant height is observed for control i.e no urea application (40.30cm in Khanyari and 33.30 cm in G M Dari). As compared to the soil application of recommended dose of urea(T_1), there is 18.1% increase in plant height in Khanyari and 11.9% in G M Dari respectively using foliar spray of 125 ppm concentration of nano-urea.

4.1.2 Effect of nano-urea on number of leaves

The perusal of data (Table 4.1) reveals that there is a significant difference in the number of leaves per plant due to the foliar spray of different concentrations of nano-urea on the plant. The effect of different concentrations of nano-urea is statistically significant over control (i.e, no urea application). The maximum number of leaves (13.40 for Khanyari and 11.40 for G M Dari) is observed for the treatment T_8 (125 ppm) which is followed by treatment T_7 i.e 250 ppm

(11.30 for Khanyari and 10.30 for G M dari). As compared to the soil application of recommended dose of urea (T_1), there is 29% increase in the no. of leaves in Khanyari and 27.19% in G M Dari respectively using foliar spray of 125 ppm concentration of nano-urea. The lowest number of leaves in both the varieties is observed in control i.e, no urea application (5.30 for khanyari and 4.20 for G M Dari).

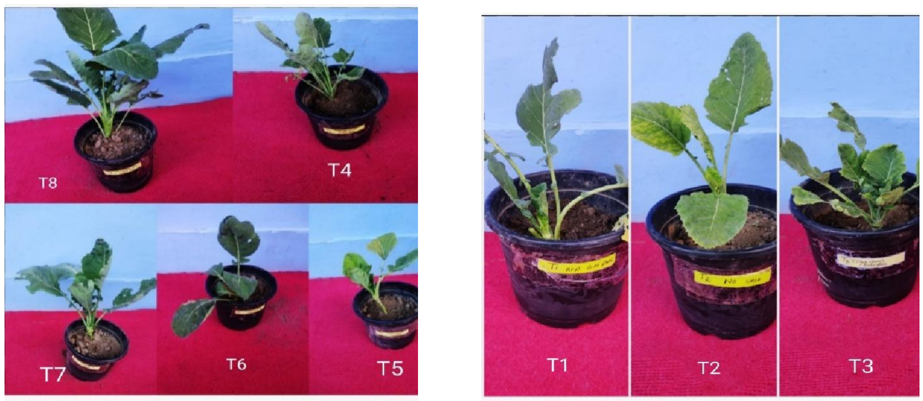
4.1.3 Effect of nano-urea on leaf area per plant (cm^2)

Significant difference in leaf area is recorded (Table 4.2) with the foliar application of nano-urea on Kale where the effect of different concentrations of nano-urea is statistically significant over control (i.e, no urea application) although the highest leaf area is recorded with application of T_8 treatment (1910.63 cm^2 in Khanyari and 1703.97cm^2 in GM Dari) which is followed by T_7 treatment (1704.23 cm^2 in Khanyari and 1512.63 cm^2 in G M Dari) . Lowest leaf area is recorded in control i.e, no urea application (551.23cm^2 in Khanyari and 501.07cm^2 in GM Dari). As compared to the soil application of recommended dose of urea recommendation(T_1), there is 13.6% increase in leaf area per plant in Khanyari and 10.7% in G M Dari respectively using foliar spray of 125 ppm concentration of nano-urea.

4.1.4 Effect of nano-urea on fresh weight of leaves (g/plant)

The data presented in (Table 4.2) reveals that T_8 proved significantly superior over all other nano-urea treatments. The fresh weight of leaves being (109.20 g/plant in Khanyari and 100.20 g/plant in GM Dari) in T_8 which is followed by T_7(89.70 g/plant in Khanyari and 84.17 g/plant in GM Dari) respectively where 250 ppm of nano-urea is used for foliar spray. As compared to the soil application of recommended dose of urea (T_1), there is 19.9% increase in fresh weight of leaves in Khanyari and 18.7% in G M Dari respectively using foliar spray of 125 ppm concentration of nano-urea.The lowest results are obtained for control i.e, no urea application (30.30 g/plant in Khanyari and 28.10 g/plant in GM Dari).

Results obtained 15 DAS in G M Dari



Results obtained in G M Dari at harvesting stage

Results obtained 15 DAS in Khanyari

Results observed in Khanyari at harvesting stage

Plate 4: Results obtained at 15 DAS and at harvesting stage in both the varieties of Kale.

Table 4.1: Effect of nano-urea on plant height (cm) and number of leaves in Kale

Treatments	Plant height (cm)			Number of leaves		
	Khanyari	GM Dari	Mean	Khanyari	GM Dari	Mean
Recommended soil application (T_1)	72.40	42.45	57.42	9.30	8.30	8.80
No urea or control (T_2)	40.30	33.30	36.80	5.30	4.20	4.75
urea spray 2000 ppm (T_3)	44.50	36.50	40.50	6.20	5.10	5.65
nano-urea spray 2000 ppm (T_4)	48.60	37.50	43.05	7.20	6.20	6.70
nano-urea spray 1000 ppm (T_5)	59.20	39.20	49.20	8.30	7.20	7.75
nano-urea spray 500 ppm (T_6)	69.80	42.20	55.50	9.20	8.10	8.65
nano-urea spray 250 ppm (T_7)	75.30	45.20	60.25	11.30	10.30	10.80
nano-urea spray 125 ppm (T_8)	88.50	48.20	68.35	13.40	11.40	12.40
Mean	62.32	40.56		8.77	7.60	
	C.D ($p\leq0.05$) Variety: **2.67** Treatment: **2.31** Variety×Treatment: **4.98**			**C.D ($p\leq0.05$)** Variety: **0.59** Treatment: **1.18** Variety×Treatment: **1.77**		

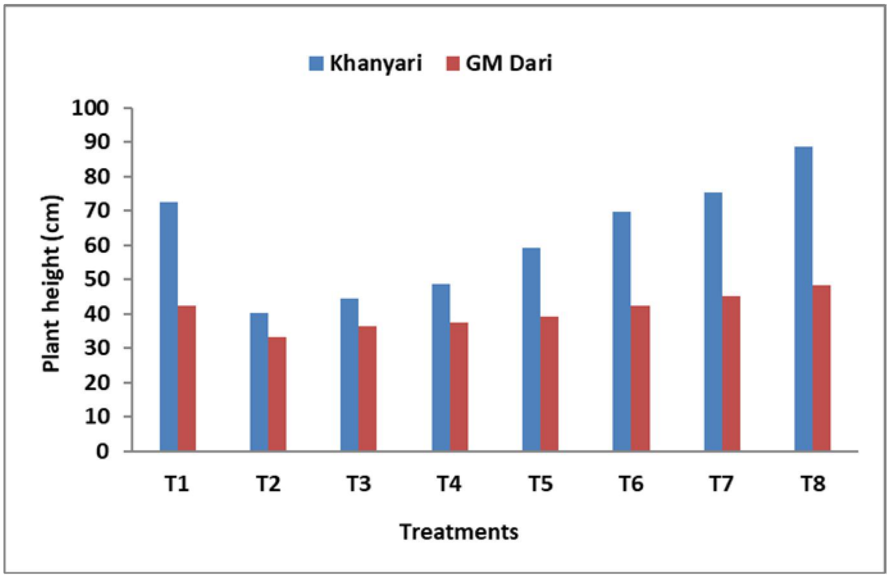


Fig. 2: Graphical representation of effect of nano-urea on plant height in Kale

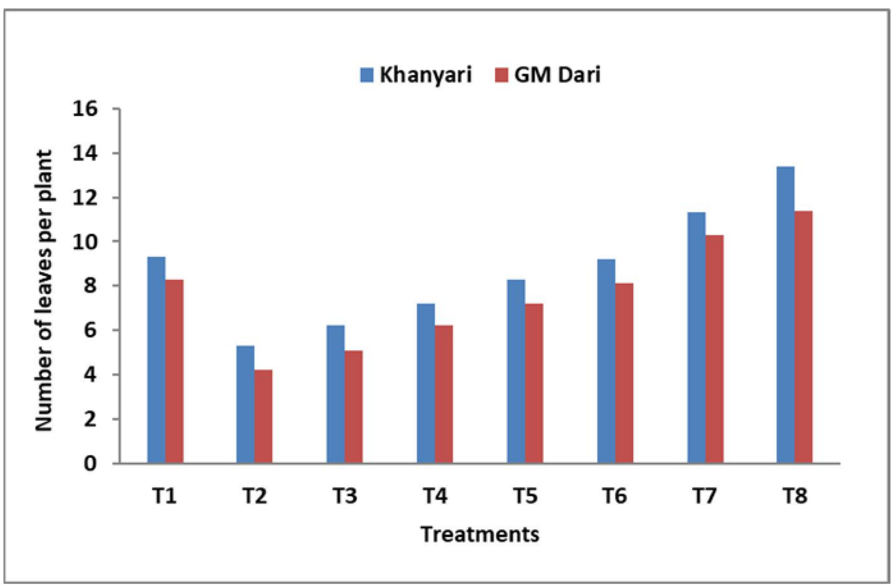


Fig. 3: Graphical representation of effect of nano-urea on number of leaves per plant in Kale

Table 4.2: Effect of nano-urea on leaf area per plant (cm^2) and fresh weight of leaves (g/plant) in Kale

Treatments	Leaf area per plant (cm^2)			Fresh weight of leaves (g/plant)		
	Khanyari	**GM Dari**	**Mean**	**Khanyari**	**GM Dari**	**Mean**
Recommended soil application (T_1)	1650.57	1520.01	1585.29	86.90	81.10	84.00
No urea or control (T_2)	551.23	501.07	526.15	30.30	28.10	29.20
urea spray 2000 ppm (T_3)	763.31	702.63	732.97	40.10	37.20	38.65
nano-urea spray 2000 ppm (T_4)	770.30	705.97	738.13	44.20	41.30	42.75
nano-urea spray 1000 ppm (T_5)	1055.71	911.53	983.62	51.30	49.20	50.25
nano-urea spray 500 ppm (T_6)	1445.67	1203.01	1324.34	69.30	60.10	64.70
nano-urea spray 250 ppm (T_7)	1704.23	1512.63	1608.43	89.70	84.17	86.93
nano-urea spray 125 ppm (T_8)	1910.63	1703.97	1807.30	109.20	100.20	104.70
Mean	1231.45	1095.10		65.12	60.17	
	C.D ($p \leq 0.05$) Variety: **110.91** Treatment: **245.80** Variety×Treatment: **368.71**			**C.D ($p \leq 0.05$)** Variety: **1.89** Treatment: **2.76** Variety×Treatment: **4.65**		

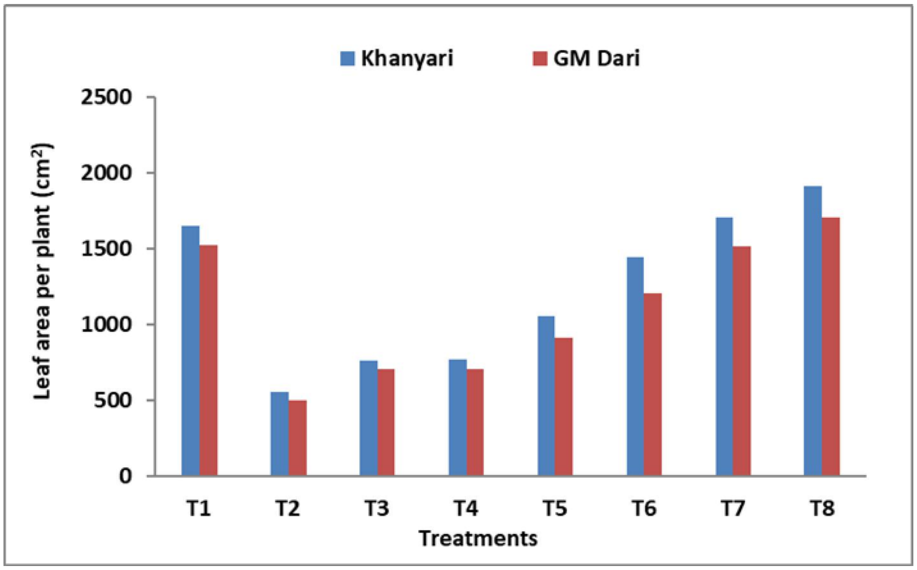


Fig. 4: Graphical representation of effect of nano-urea on leaf area per plant in Kale

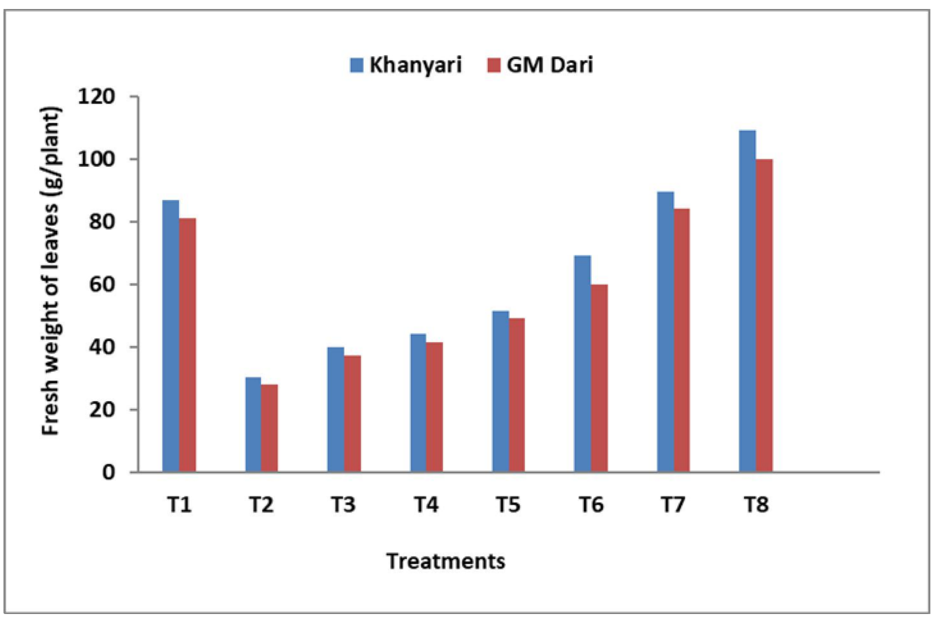


Fig. 5: Graphical representation of effect of nano-urea on fresh weight of leaves in Kale

4.1.5 Effect of nano-urea on dry weight of leaves (g/plant)

The data in Table 4.3 depicts variations in dry weight of leaves due to different concentrations of nano-urea sprays. The results reveal that as the concentration of nano-urea is decreased, the dry weight of leaves increases. The best results are seen with T_8 which is followed by T_7 in both the varieties. The maximum dry weight of leaves being (14.70 g/plant in Khanyari and 13.20 g/plant in GM Dari) in T_8 where the concentration of nano-urea used is 125 ppm and (11.50 g/plant in Khanyari and 10.30 g/plant in GM Dari) in T_7 where the concentration of nano-urea used is 250 ppm respectively. The lowest results are obtained in control i.e, no urea application (5.20 g/plant in Khanyari and 4.30 g/plant in GM Dari). As compared to the soil application of recommended dose of urea (T_1), there is 27% increase in dry weight of leaves in Khanyari and 27.6% in G M Dari respectively using foliar spray of 125 ppm of nano-urea.

4.2 Physiological parameters

4.2.1 Effect of nano-urea on net photosynthetic rate (µmole m^{-2} sec^{-1})

The perusal of data in Table 4.3 reveals that there is an increase in net photosynthetic rate due to various nano-urea treatments over control i.e no urea application, however the treatment T_8 proved significantly superior over all other nano-urea treatments followed by T_7 treatment in both the varieties. The net photosynthetic rate is (17.20 µmole m^{-2} sec^{-1} in Khanyari and 15.40 µmole m^{-2} sec^{-1} in G M Dari) in T_8 where the concentration of nano-urea used is 125 ppm and (16.20 µmole m^{-2} sec^{-1} in Khanyari and 14.20 µmole m^{-2} sec^{-1} in G M Dari) in T_7, where the concentration of nano-urea used is 250 ppm respectively. As compared to the soil application of recommended dose of urea (T_1), there is 10.7% increase in net photosynthetic rate in Khanyari and 13.9% in G M Dari respectively using foliar spray of 125 ppm concentration of nano-urea. The lowest results are obtained in control i.e, no urea application (11.30 µmole m^{-2} sec^{-1} in Khanyari and 9.20 µmole m^{-2} sec^{-1} in GM Dari).

4.2.2 Effect of nano-urea on stomatal conductance (mmole m^{-2} sec^{-1})

The data on stomatal conductance in both the varieties of Kale (Khanyari and GM Dari) is presented in Table 4.4. The data indicates that the stomatal conductance is markedly influenced by foliar spray of nano-urea where the effect of different concentrations of nano-urea was statistically significant over control (i.e, no urea application). There is a significant difference in stomatal conductance due to the application of nano-urea treatments of varied concentrations. However, maximum stomatal conductance (0.51 mmole m^{-2} sec^{-1} in Khanyari and 0.40 mmole m^{-2} sec^{-1} in GM Dari) is observed for the treatment T_8 where the concentration of nano-urea is 125 ppm which is followed by T_7 treatment (0.46 mmole m^{-2} sec^{-1} in Khanyari and 0.38 mmole m^{-2} sec^{-1} in GM Dari) where the concentration of nano-urea is 250 ppm. The lowest value is observed for control i.e, no urea application (0.24 mmole m^{-2} sec^{-1} in Khanyari and 0.22 mmole m^{-2} sec^{-1} in GM Dari). As compared to the soil application of recommended dose of urea (T_1), there is 17.6% increase in stomatal conductance in Khanyari and 10% in G M Dari respectively using foliar spray of 125 ppm concentration of nano-urea.

4.2.3 Effect of nano-urea on transpiration rate (µmole m^{-2} sec^{-1})

The effect of nano-urea (Table 4.4) on transpiration rate in both the varieties of Kale (Khanyari and GM Dari) is statistically significant over control (i.e, no urea application). The data indicates that the transpiration rate is markedly influenced by foliar spray of nano-urea. However, maximum transpiration rate (5.71µmole m^{-2} sec^{-1} in Khanyari and 4.70 µmole m^{-2} sec^{-1} in G M Dari) is observed for the treatment T_8 where the concentration of nano-urea used is 125 ppm which is followed by T_7 treatment (5.11µmole m^{-2} sec^{-1} in Khanyari and 4.29 µmole m^{-2} sec^{-1} in GM Dari) where the concentration of nano-urea is 250 ppm. As compared to the soil application of recommended dose of urea (T_1), there is 17.1% increase in transpiration rate in Khanyari and 17.7% in G M Dari respectively using foliar spray of 125 ppm concentration of nano-urea. The lowest value is observed for control i.e, no urea application (3.41µmole m^{-2} sec^{-1} in Khanyari and 2.40 µmole m^{-2} sec^{-1} in G M Dari).

Table 4.3: Effect of nano-urea on dry weight of leaves (g/plant) and net photosynthetic rate (µmole m^{-2} sec^{-1}) in Kale

Treatments	Dry weight of leaves (g/plant)			Net photosynthetic rate (µmole m^{-2} sec^{-1})		
	Khanyari	**GM Dari**	**Mean**	**Khanyari**	**GM Dari**	**Mean**
Recommended soil application (T_1)	10.60	9.50	10.05	15.45	13.25	14.35
No urea or control (T_2)	5.20	4.30	4.75	11.30	9.20	10.25
urea spray 2000 ppm (T_3)	6.40	5.60	6.00	13.30	11.10	12.10
nano-urea spray 2000 ppm (T_4)	6.80	5.80	6.30	13.40	11.40	12.40
nano-urea spray 1000 ppm (T_5)	8.30	7.20	7.75	14.30	12.10	13.20
nano-urea spray 500 ppm (T_6)	9.30	8.30	8.80	15.30	13.10	14.20
nano-urea spray 250 ppm (T_7)	11.50	10.30	10.90	16.20	14.20	15.20
nano-urea spray 125 ppm (T_8)	14.70	13.20	13.95	17.20	15.40	16.30
Mean	9.10	8.03		14.55	12.46	
	C.D ($p \leq 0.05$) Variety: **0.59** Treatment: **1.18** Variety×Treatment: **1.77**			**C.D ($p \leq 0.05$)** Variety: **1.12** Treatment: **1.98** Variety×Treatment: **3.10**		

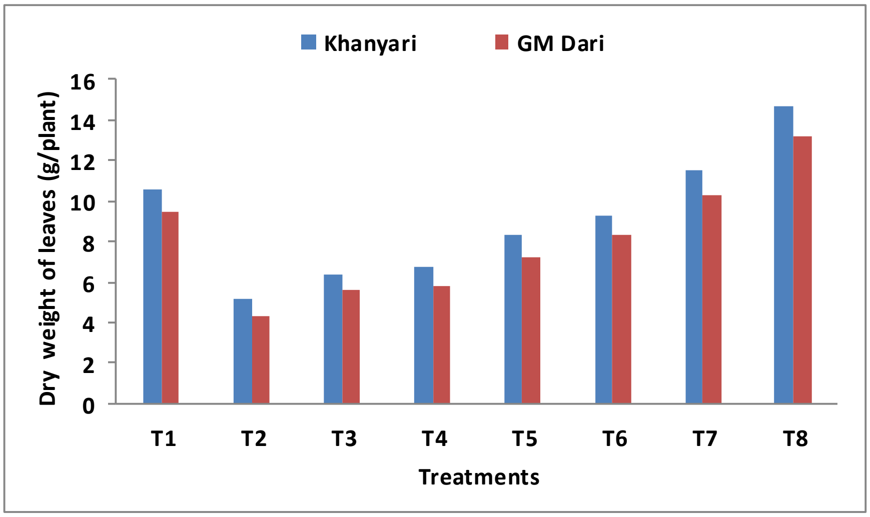


Fig. 6: Graphical representation of effect of nano-urea on dry weight of leaves in Kale

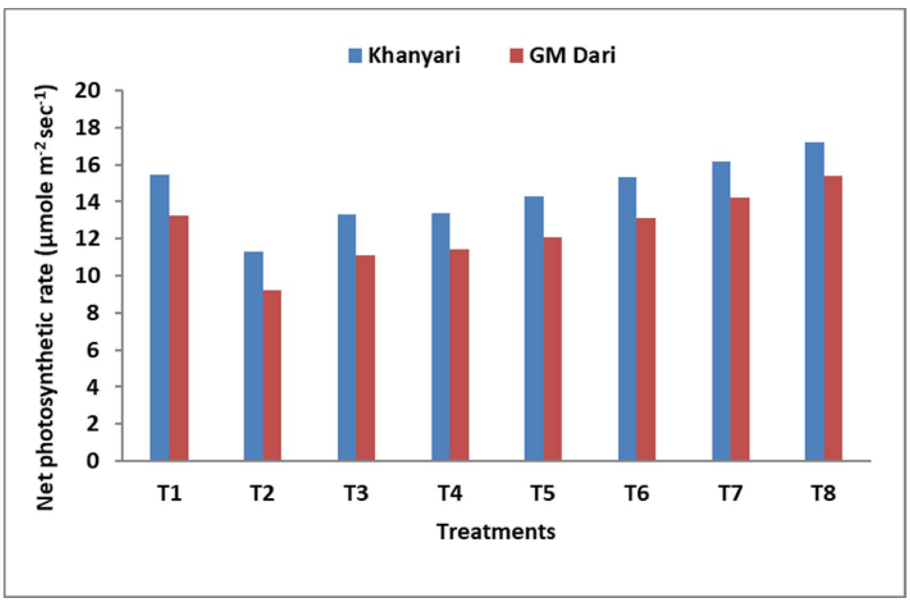


Fig. 7: Graphical representation of effect of nano-urea on net photosynthetic rate in Kale

Table 4.4: Effect of nano-urea on stomatal conductance (mmole m^{-2} sec^{-1}) and transpiration rate (µmole m^{-2} sec^{-1}) in Kale

Treatments	Stomatal conductance (mmole m^{-2} sec^{-1})			Transpiration rate (µmole m^{-2} sec^{-1})		
	Khanyari	**GM Dari**	**Mean**	**Khanyari**	**GM Dari**	**Mean**
Recommended soil application (T_1)	0.42	0.36	0.39	4.73	3.87	4.30
No urea or control (T_2)	0.24	0.22	0.24	3.41	2.47	2.91
urea spray 2000 ppm (T_3)	0.30	0.26	0.28	4.18	3.15	3.66
nano-urea spray 2000 ppm (T_4)	0.33	0.28	0.31	4.21	3.25	3.73
nano-urea spray 1000 ppm (T_5)	0.38	0.31	0.35	4.44	3.55	4.00
nano-urea spray 500 ppm (T_6)	0.41	0.35	0.38	4.71	3.84	4.28
nano-urea spray 250 ppm (T_7)	0.46	0.38	0.42	5.11	4.29	4.70
nano-urea spray 125 ppm (T_8)	0.51	0.40	0.45	5.71	4.70	5.20
Mean	0.38	0.36		4.56	3.64	
	C.D ($p \leq 0.05$) Variety: **0.01** Treatment: **0.10** Variety×Treatment: **0.11**			**C.D ($p \leq 0.05$)** Variety: **0.78** Treatment: **1.36** Variety×Treatment: **2.14**		

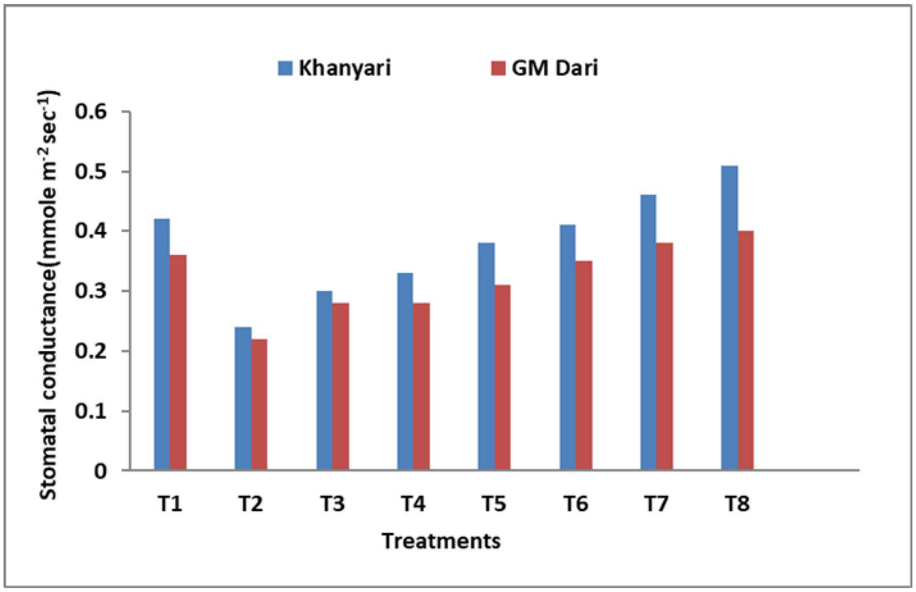


Fig.8: Graphical representation of effect of nano-urea on stomatal conductance in Kale

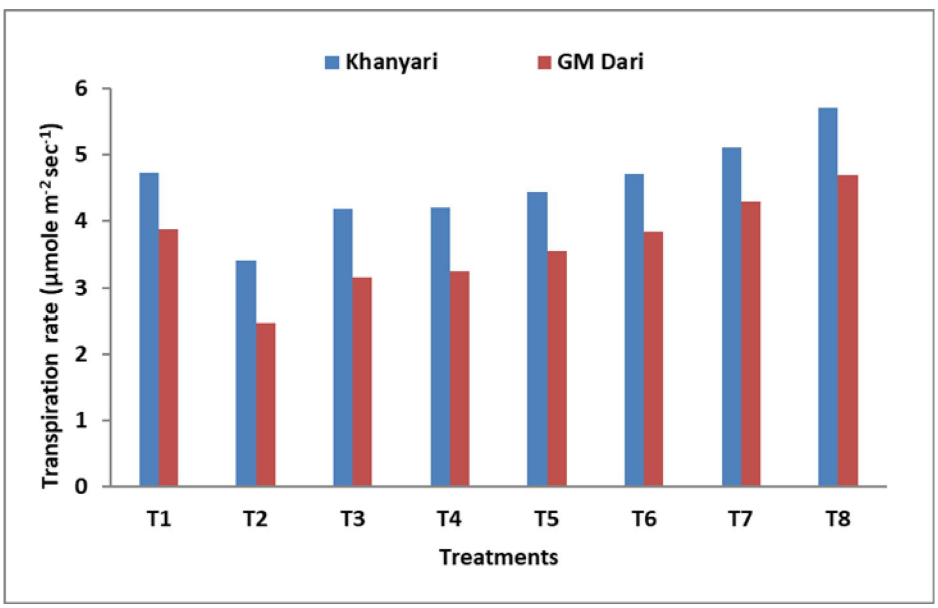


Fig. 9: Graphical representation of effect of nano-urea on transpiration rate in Kale

4.2.4 Effect of nano-urea on leaf chlorophyll a content (mg g^{-1} FW)

The leaf chlorophyll a content of the G M Dari and Khanyari cultivars of Kale is shown in Table 4.5. Information reveals that the level of chlorophyll a in the leaves is significantly affected by the foliar application of nano-urea. There is a significant increase in chlorophyll a content by increasing the concentration of nano-urea. Maximum leaf chlorophyll a content (1.45 mg g^{-1} FW in Khanyari and 1.28 mg g^{-1} FW in GM Dari) is observed for the treatment T_4 where the concentration of nano-urea is 2000 ppm which is followed by T_3 treatment (1.42 mg g^{-1} FW in Khanyari and 1.26 mg g^{-1} FWin GM Dari)where the concentration of urea spray is 2000 ppm. The lowest value is observed for control i.e, no urea application (1.02 mg g^{-1} FW in Khanyari and 0.91 mg g^{-1} FW in GM Dari).). As compared to the soil application of recommended dose of urea(T_1), there is 11.7% increase in chlorophyll a content in Khanyari and 3.6% in G M Dari respectively using foliar spray of 125 ppm concentration of nano-urea.

4.2.5 Effect of nano-urea on leaf chlorophyll b content (mg g^{-1} FW)

The data on leaf chlorophyll b content in both the varieties of Kale (Khanyari and G M Dari) is presented in Table 4.5. The data indicates that the leaf chlorophyll b content is markedly influenced by foliar spray of nano-urea. There is a significant increase in chlorophyll b content by increasing the concentration of nano-urea. However, maximum value (0.71 mg g^{-1} FW in Khanyari and 0.56 mg g^{-1} FW in GM Dari) is observed for the treatment T_4 where the concentration of nano-urea is 2000 ppm which is followed by T_3 treatment (0.67 mg g^{-1} FW in Khanyari and 0.54 mg g^{-1} FW in GM Dari) where the concentration of urea spray is 2000 ppm. As compared to the soil application of recommended dose of urea(T_1), there is 18.4% increase in chlorophyll b content in Khanyari and 17.14% in G M Dari respectively using foliar spray of 125 ppm concentration of nano-urea. The lowest value is observed for control i.e, no urea application (0.23 mg g^{-1} FW in Khanyari and 0.21 mg g^{-1} FW in GM Dari).

Table 4.5: Effect of nano-urea on leaf chlorophyll a (mg g^{-1} FW) and leaf chlorophyll b (mg g^{-1} FW) content in Kale

Treatments	Leaf chlorophyll a (mg g^{-1} FW)			Leaf chlorophyll b (mg g^{-1} FW)		
	Khanyari	GM Dari	Mean	Khanyari	GM Dari	Mean
Recommended soil application (T_1)	1.13	1.05	1.09	0.31	0.29	0.33
No urea or control (T_2)	1.02	0.91	0.97	0.23	0.21	0.22
urea spray 2000 ppm (T_3)	1.42	1.26	1.34	0.67	0.54	0.61
nano-urea spray 2000 ppm (T_4)	1.45	1.28	1.37	0.71	0.56	0.64
nano-urea spray 1000 ppm (T_5)	1.41	1.23	1.32	0.61	0.51	0.56
nano-urea spray 500 ppm (T_6)	1.34	1.14	1.24	0.52	0.45	0.48
nano-urea spray 250 ppm (T_7)	1.31	1.11	1.21	0.43	0.41	0.42
nano-urea spray 125 ppm (T_8)	1.28	1.09	1.18	0.38	0.35	0.37
Mean	1.25	1.10		0.44	0.39	
	C.D ($p \leq 0.05$) Variety: **0.06** Treatment: **0.12** Variety×Treatment: **0.18**			**C.D ($p \leq 0.05$)** Variety: **0.006** Treatment: **0.012** Variety×Treatment: **0.018**		

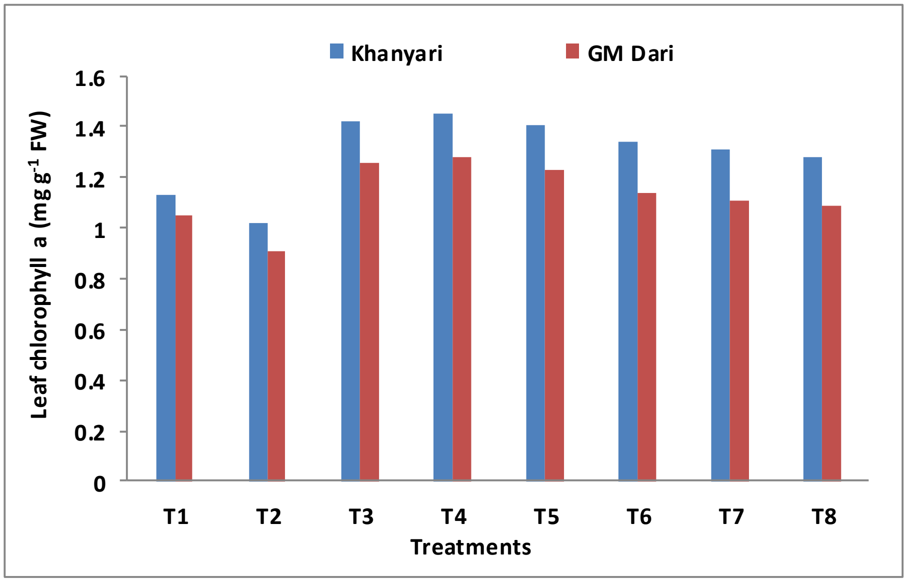


Fig.10: Graphical representation of effect of nano-urea on leaf chlorophyll a content in Kale

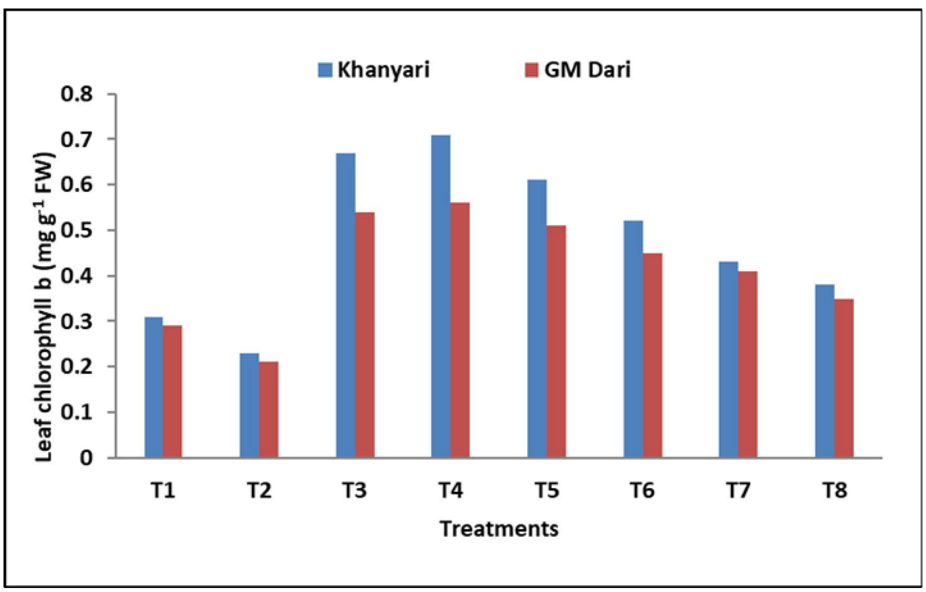


Fig.11: Graphical representation of effect of nano-urea on leaf chlorophyll b content in Kale

4.2.6 Effect of nano-urea on total leaf chlorophyll content (mg g^{-1} FW)

The perusal of data (Table 4.6) reveals that the total leaf chlorophyll content in both the varieties of Kale (Khanyari and GM Dari) is markedly influenced by the foliar spray of nano-urea. A significant increase in total leaf chlorophyll content is seen with increasing the concentration of nano-urea. However, the maximum value (2.16 mg g^{-1} FW in Khanyari and 1.85 mg g^{-1} FW in GM Dari) is observed for the treatment T_4 where the concentration of nano-urea used is 2000 ppm which is followed by T_3 treatment (2.07 mg g^{-1} FW in Khanyari and 1.80 mg g^{-1} FW in GM Dari) where the concentration of urea spray is 2000 ppm . The lowest value for total leaf chlorophyll content is observed for control i.e, no urea application (1.29 mg g^{-1} FW in Khanyari and 1.12 mg g^{-1} FW in GM Dari). As compared to the soil application of recommended dose of urea (T_1), there is 10.3% increase in total leaf chlorophyll content in Khanyari and 6% in G M Dari respectively using foliar spray of 125 ppm concentration of nano-urea. A higher dose of nano-urea (2000 ppm) promotes the formation of maximum amount of chlorophyll in the leaves which otherwise is a toxic concentration with respect to growth and other quality parameters of the plant.

4.2.7 Effect of nano-urea on leaf carotenoid content (mg g^{-1} FW)

The analysis of the data (Table 4.6) shows that the foliar application of nano-urea has a significant impact on the leaf carotenoid content in both the Khanyari and GM Dari cultivars of Kale. When the amount of nano-urea in the spray solution is increased, there is a noticeable rise in the amount of carotenoid content in the leaves. However, the maximum value (0.35 mg g^{-1} FW in Khanyari and 0.33 mg g^{-1}FW in GM Dari) is observed for the treatment T_4 where the concentration of nano-urea is 2000 ppm which is followed by T_3 treatment (0.33 mg g^{-1} FW in Khanyari and 0.29 mg g^{-1}FW in GM Dari), where the concentration of urea spray is 2000 ppm. As compared to the soil application of the recommended dose of urea(T_1), there is 11.1% increase in leaf carotenoid content in Khanyari and 12% in G M Dari respectively using foliar spray of 125 ppm concentration of nano-urea. The lowest value is observed for control i.e, no urea application (0.21 mg g^{-1} FW in Khanyari and 0.19 mg g^{-1} FW in GM Dari).

Table 4.6: Effect of nano-urea on total leaf chlorophyll content (mg g^{-1} FW) and leaf carotenoid content (mg g^{-1} FW) in Kale

Treatments	Total leaf chlorophyll (mg g^{-1} FW)			Leaf carotenoid content (mg g^{-1} FW)		
	Khanyari	**GM Dari**	**Mean**	**Khanyari**	**GM Dari**	**Mean**
Recommended soil application (T_1)	1.48	1.40	1.44	0.24	0.22	0.24
No urea or control (T_2)	1.29	1.12	1.21	0.21	0.19	0.20
urea spray 2000 ppm (T_3)	2.07	1.80	1.94	0.33	0.29	0.30
nano-urea spray 2000 ppm (T_4)	2.16	1.85	2.01	0.35	0.33	0.34
nano-urea spray 1000 ppm (T_5)	2.02	1.75	1.89	0.32	0.30	0.32
nano-urea spray 500 ppm (T_6)	1.87	1.61	1.74	0.31	0.28	0.29
nano-urea spray 250 ppm (T_7)	1.74	1.56	1.65	0.29	0.27	0.27
nano-urea spray 125 ppm (T_8)	1.65	1.49	1.57	0.27	0.25	0.26
Mean	1.71	1.51		0.28	0.25	
	C.D ($p \leq 0.05$) Variety: **0.01** Treatment**: 0.02** Variety×Treatment: **0.03**			**C.D ($p \leq 0.05$)** Variety: **0.024** Treatment: **0.092** Variety×Treatment: **0.121**		

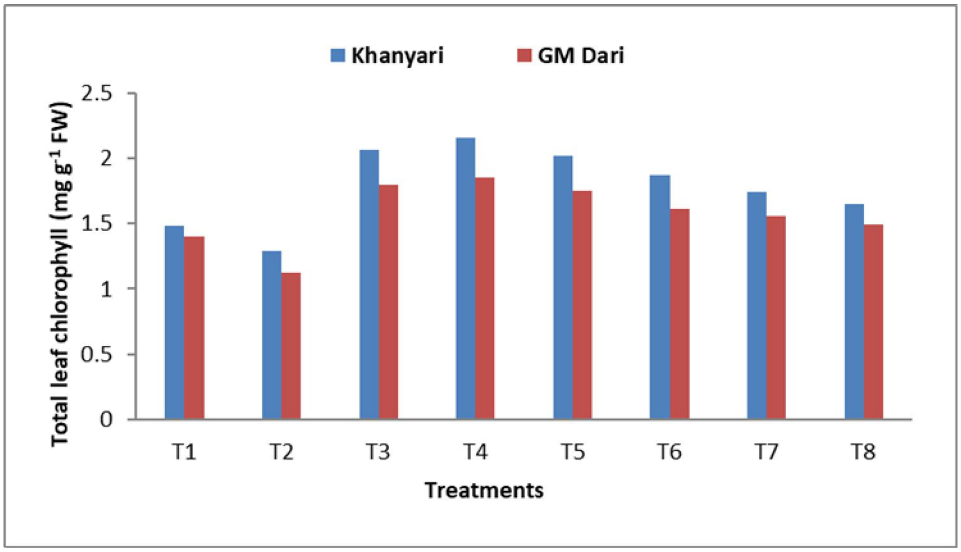


Fig.12: Graphical representation of effect of nano-urea on total leaf chlorophyll content in Kale

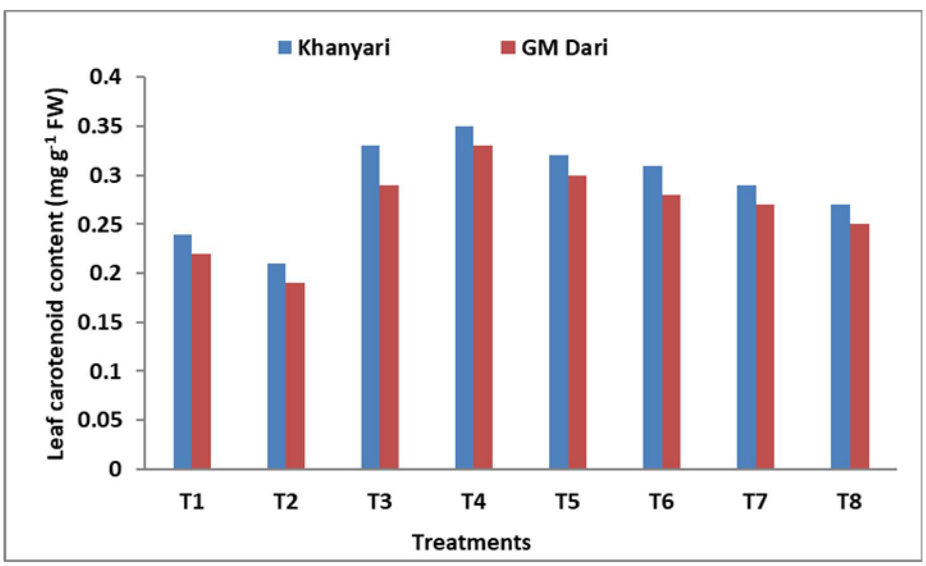


Fig.13: Graphical representation of effect of nano-urea on leaf carotenoid content in Kale

4.2.8 Effect of nano-urea on net assimilation rate (g m^{-2} day^{-1})

The data on net assimilation rate during (0-20 days and 20-40 days) in both the varieties of Kale (Khanyari and GM Dari) is presented in Table 4.7. The data indicates that the net assimilation rate at both the time periods is markedly influenced by foliar spray of nano urea. There is a significant difference in net assimilation rate due to the nano urea treatments of varied concentrations. However, maximum value for both the time periods (3.71 g m^{-2} day^{-1} for 0-20 days, 4.96 g m^{-2} day^{-1} for 20-40 days in Khanyari and 2.81 g m^{-2} day^{-1} for 0-20 days, 4.11 g m^{-2} day^{-1} for 20-40 days in GM Dari) is observed for the treatment T_8 where the concentration of nano-urea is 125 ppm which is followed by T_7 treatment (3.41 g m^{-2} day^{-1} for 0-20 days, 4.71 g m^{-2} day^{-1} for 20-40 days in Khanyari and 2.51 g m^{-2} day^{-1} for 0-20 days, 3.97 g m^{-2} day^{-1} for 20-40 days in GM Dari) where the concentration of nano-urea is 250 ppm . The lowest value is observed for control i.e, no urea application (1.22 g m^{-2} day^{-1} for 0-20 days, 2.22 g m^{-2} day^{-1} for 20-40 days in Khanyari and 1.13 g m^{-2} $day^{-1}$for 0-20 days, 1.64 g m^{-2} day^{-1} for 20-40 days in G M Dari). A decrease in net assimilation rate is seen with increasing the concentration of nano-urea. Concentration as high as 2000 ppm for both nano-urea and urea solution has more negative impact on the net assimilation rate and lead to a greater decrease in the plant growth. As compared to the soil application of the recommended dose of urea(T_1), there is 13.7% increase in net assimilation rate in Khanyari and 18.1% in G M Dari respectively using foliar spray of 125 ppm concentration of nano-urea.

Table 4.7: Effect of nano-urea on net assimilation rate, NAR (g m^{-2} day^{-1}) in Kale

Treatments	Net assimilation rate,NAR (g m^{-2} day^{-1})					
	NAR A (0-20 days)			NAR B (20-40 days)		
	Khanyari	GM Dari	Mean	Khanyari	GM Dari	Mean
Recommended soil application (T_1)	3.20	2.30	2.75	4.50	3.70	4.10
No urea or control (T_2)	1.22	1.13	1.18	2.22	1.64	1.93
urea spray 2000 ppm (T_3)	2.31	1.51	1.91	3.42	2.40	2.91
nano-urea spray 2000 ppm (T_4)	2.33	1.53	1.93	3.45	2.46	3.26
nano-urea spray 1000 ppm (T_5)	2.71	1.81	2.26	4.21	3.31	3.76
nano-urea spray 500 ppm (T_6)	3.11	2.22	2.67	4.40	3.61	4.01
nano-urea spray 250 ppm (T_7)	3.41	2.51	2.96	4.71	3.97	4.34
nano-urea spray 125 ppm (T_8)	3.71	2.81	3.26	4.96	4.11	4.53
Mean	2.75	1.97		3.98	3.15	
	C.D ($p \leq 0.05$) Variety: **0.73** Treatment: **1.39** Variety×Treatment: **2.12**			**C.D ($p \leq 0.05$)** Variety: **0.55** Treatment: **0.98** Variety×Treatment: **1.53**		

4.2.9 Effect of nano-urea on relative growth rate ($g\ g^{-1}\ day^{-1}$)

Table 4.8 shows the information on the relative growth rate during the course of 0–20 and 20–40 days for both the Khanyari and GM Dari kinds of Kale. The data shows that the application of foliar nano-urea has a significant impact on the relative growth rate at both time periods. There is a significant difference in relative growth rate due to the nano-urea treatments of varied concentrations. However, maximum value for both the time periods (0.71 g $g^{-1}day^{-1}$ for 0-20 days, 0.85 g $g^{-1}day^{-1}$ for 20-40 days in Khanyari and 0.62 g $g^{-1}day^{-1}$ for 0-20 days, 0.74 g $g^{-1}day^{-1}$for 20-40 days in GM Dari) is observed for the treatment T_8 where the concentration of nano-urea is 125 ppm which is followed by T_7 treatment (0.61 g $g^{-1}day^{-1}$ for 0-20 days, 0.72 g $g^{-1}day^{-1}$ for 20-40 days in Khanyari and 0.56 g $g^{-1}day^{-1}$ for 0-20 days, 0.66 g $g^{-1}day^{-1}$for 20-40 days in GM Dari) where the concentration of nano-urea is 250 ppm . The lowest value is observed for control i.e, no urea application (0.31 g $g^{-1}day^{-1}$ for 0-20 days, 0.41 g $g^{-1}day^{-1}$ for 20-40 days in Khanyari and 0.28 g $g^{-1}day^{-1}$ for 0-20 days, 0.32 g $g^{-1}day^{-1}$ for 20-40 days in GM Dari) . As compared to the soil application of the recommended dose of urea(T_1), there is 19.7% increase in relative growth rate in Khanyari and 14.5% in G M Dari respectively using foliar spray of 125 ppm of nano-urea.

4.3 Biochemical parameters

4.3.1 Effect of nano-urea on total phenols (mg GAE g^{-1})

The data on total phenols in both the varieties of Kale (Khanyari and GM Dari) is presented in Table 4.9. The results show that the foliar spraying of nano-urea has a significant impact on the total phenols. There is a significant difference in total phenols due to the nano-urea treatments of varied concentrations. However, maximum value (2.06 mg GAE g^{-1} in Khanyari and 1.42 mg GAE g^{-1} in GM Dari) is observed for the treatment T_8 where the concentration of nano- urea is 125 ppm which is followed by T_7 treatment (2.02mg GAE g^{-1} in Khanyari and 1.36 mg GAE g^{-1} in GM Dari) where the concentration of nano-urea is 250 ppm .

The lowest value is observed for control i.e, no urea application (1.66 mg GAE g^{-1} in Khanyari and 1.08 mg GAE g^{-1} in GM Dari). As compared to the soil application of the recommended dose of urea(T_1), there is 14% increase in total phenols in Khanyari and 13.3% in G M Dari respectively using foliar spray of 125 ppm concentration of nano-urea.

4.3.2 Effect of nano-urea on total carbohydrates (g/100g)

Table 4.9 provides information on the total amount of carbohydrates in both the Khanyari and GM Dari varieties of Kale. The data indicates that the total carbohydrates are markedly influenced by foliar spray of nano-urea. There is a significant difference in the total carbohydrates due to the nano-urea treatments of varied concentrations. However, maximum value (5.64 g/100g in Khanyari and 5.06 g/100g in GM Dari) is observed for the treatment T_8 where the concentration of nano-urea is 125 ppm which is followed by T_7 treatment (4.92 g/100g in Khanyari and 4.63 g/100g in GM Dari) where the concentration of nano-urea is 250 ppm. As compared to the soil application of the recommended dose of urea(T_1), there is 19.9% increase in total carbohydrates in Khanyari and 15.1% in G M Dari respectively using foliar spray of 125 ppm of nano-urea. The lowest value is observed for control i.e, no urea application (2.09 g/100g in Khanyari and 2.05 g/100g in GM Dari).

Table 4.8: Effect of nano-urea on relative growth rate, RGR (g g^{-1} day^{-1}) in Kale

Treatments	Relative growth rate, RGR (g g^{-1} day^{-1})					
	RGR A (0-20 days)			RGR B (20-40 days)		
	Khanyari	GM Dari	Mean	Khanyari	GM Dari	Mean
Recommended soil application (T_1)	0.57	0.53	0.55	0.67	0.63	0.65
No urea or control (T_2)	0.31	0.28	0.30	0.41	0.32	0.37
urea spray 2000 ppm (T_3)	0.38	0.35	0.37	0.48	0.42	0.45
nano-urea spray 2000 ppm (T_4)	0.42	0.41	0.42	0.53	0.50	0.52
nano-urea spray 1000 ppm (T_5)	0.47	0.45	0.46	0.58	0.55	0.57
nano-urea spray 500 ppm (T_6)	0.53	0.51	0.52	0.65	0.61	0.63
nano-urea spray 250 ppm (T_7)	0.61	0.56	0.59	0.72	0.66	0.69
nano-urea spray 125 ppm (T_8)	0.71	0.62	0.67	0.85	0.74	0.77
Mean	0.50	0.46		0.61	0.55	
	C.D ($p \leq 0.05$) Variety: **0.006** Treatment: **0.012** Variety×Treatment: **0.017**			**C.D ($p \leq 0.05$)** Variety**: 0.023** Treatment: **0.084** Variety×Treatment: **0.107**		

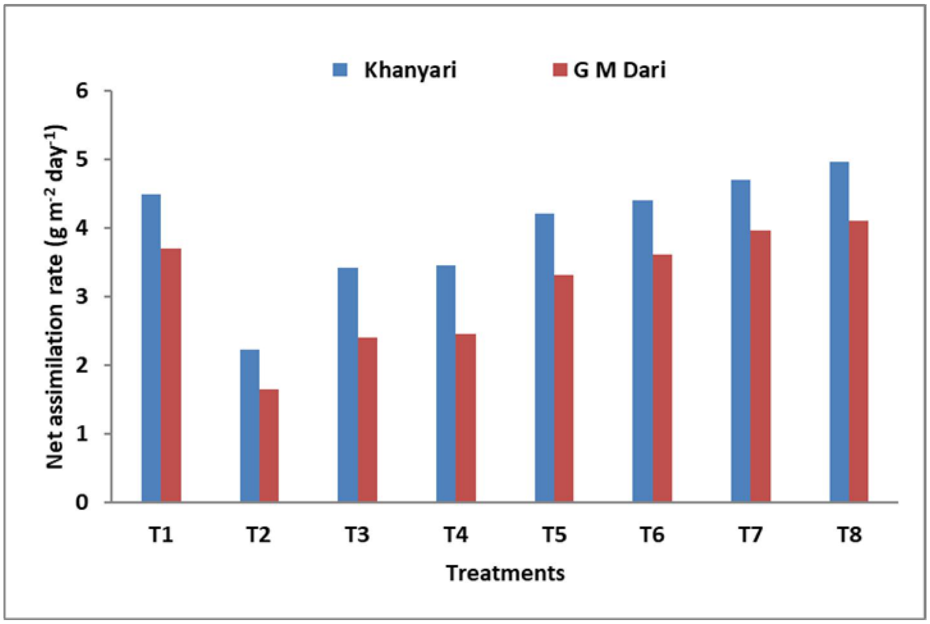


Fig.14: Graphical representation of effect of nano-urea on net assimilation rate in Kale

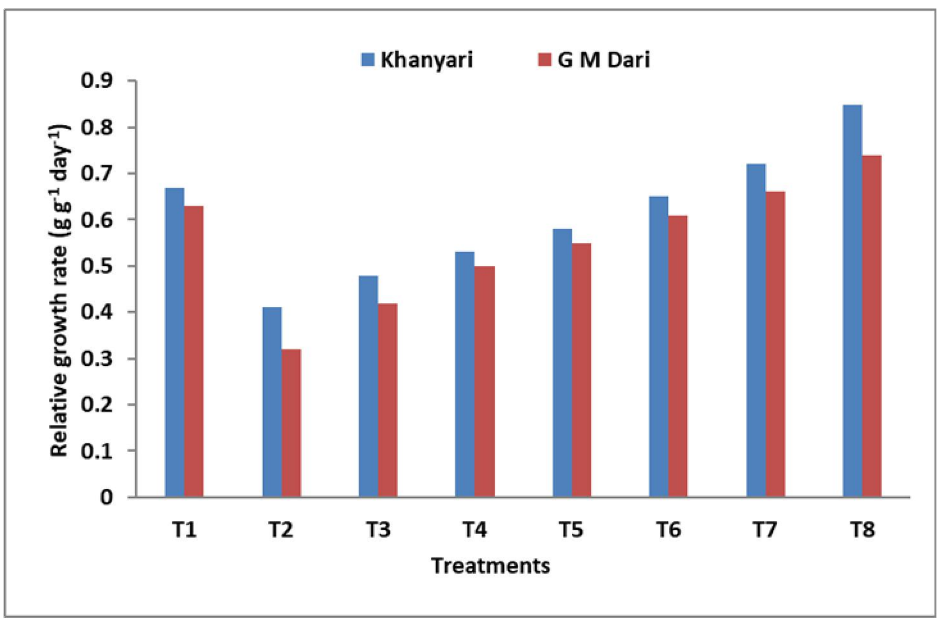


Fig.15: Graphical representation of effect of nano-urea on relative growth rate in Kale

Table 4.9: Effect of nano-urea on total phenols (mg GAE g^{-1}) and total carbohydrates (g/100g) in Kale

Treatments	Total phenols (mg GAE g^{-1})			Total carbohydrates (g/100g)		
	Khanyari	GM Dari	Mean	Khanyari	GM Dari	Mean
Recommended soil application (T_1)	1.96	1.31	1.63	4.50	4.30	4.40
No urea or control (T_2)	1.66	1.08	1.37	2.09	2.05	2.07
urea spray 2000 ppm (T_3)	1.70	1.15	1.44	3.14	3.09	3.12
nano-urea spray 2000 ppm (T_4)	1.72	1.17	1.45	3.21	3.12	3.17
nano-urea spray 1000 ppm (T_5)	1.81	1.25	1.53	3.82	3.61	3.72
nano-urea spray 500 ppm (T_6)	1.94	1.29	1.62	4.23	4.12	4.18
nano-urea spray 250 ppm (T_7)	2.02	1.36	1.69	4.92	4.63	4.78
nano-urea spray 125 ppm (T_8)	2.06	1.42	1.74	5.64	5.06	5.35
Mean	1.85	1.25		3.94	3.74	
	C.D ($p \leq 0.05$) Variety: **0.009** Treatment: **0.03** Variety×Treatment: **0.039**			**C.D ($p \leq 0.05$)** Variety: **0.05** Treatment: **0.32** Variety×Treatment: **0.37**		

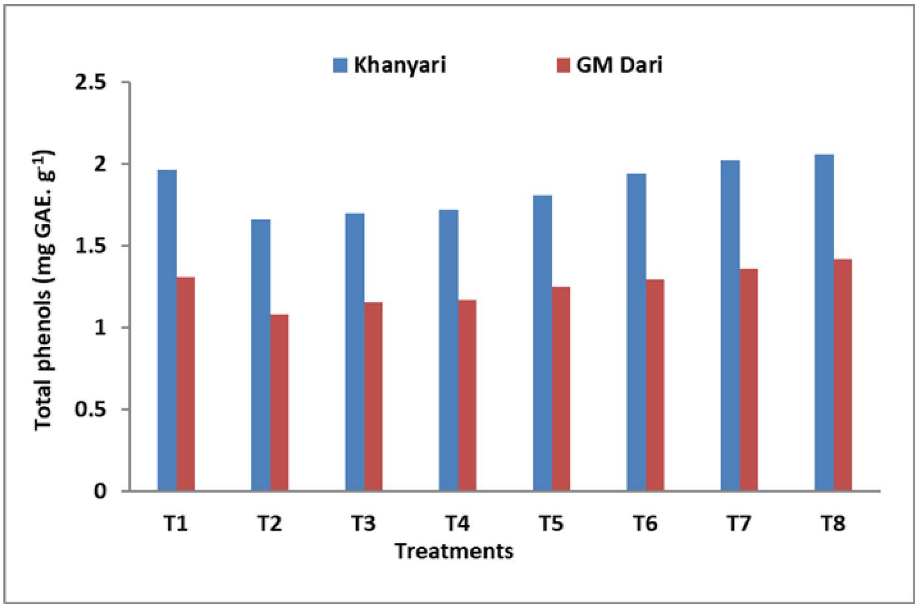


Fig.16: Graphical representation of effect of nano-urea on total phenols in Kale

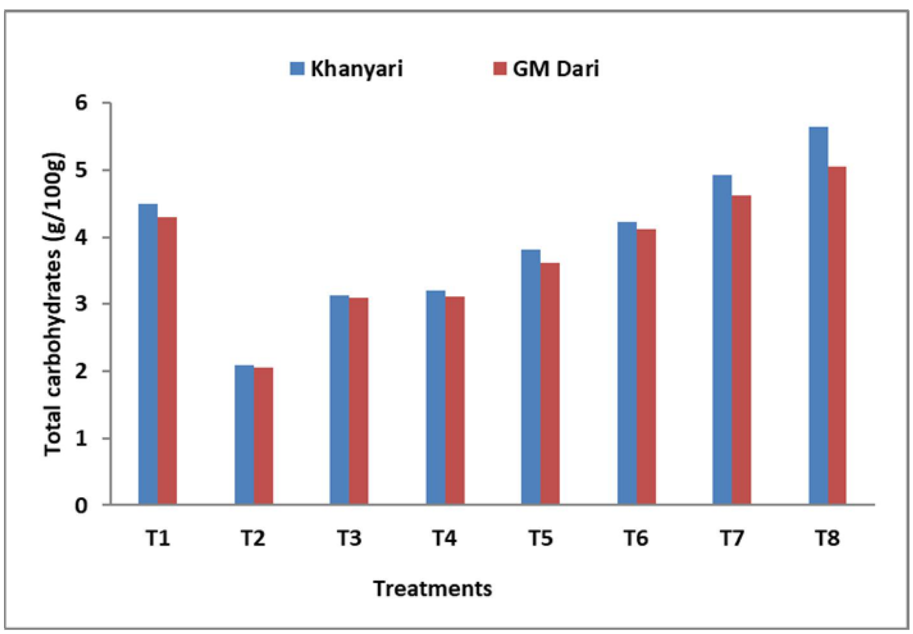


Fig.17: Graphical representation of effect of nano-urea on total carbohydrates in Kale

4.3.3 Effect of nano-urea on leaf nitrogen content (%)

The total amount of leaf nitrogen in both the Khanyari and GM Dari cultivars of Kale is shown in Table 4.10. The data shows that foliar spraying of nano-urea has a significant impact on the leaf nitrogen content. The data indicates that the leaf nitrogen content is markedly influenced by foliar spray of nano-urea. There is a significant increase in leaf nitrogen content by increasing the concentration of nano-urea liquid in the spray solution. However, maximum leaf nitrogen content (2.97% in Khanyari and 2.11% in GM Dari) is observed for the treatment T_4 where the concentration of nano-urea is 2000 ppm which is followed by T_3 treatment (2.91% in Khanyari and 1.93% in GM Dari) where the concentration of urea spray is 2000 ppm. The lowest value is observed for control i.e, no urea application (1.06% in Khanyari and 1.03% in GM Dari). With the decrease in concentration of nano-urea, the leaf nitrogen content also decreases. As compared to the soil application of the recommended dose of urea(T_1), there is 19.5% increase in the leaf nitrogen content in Khanyari and 14% in G M Dari respectively using foliar spray of 125 ppm concentration of nano-urea.

4.3.4 Effect of nano-urea on leaf phosphorus content (%)

The data on leaf phosphorus content in both the varieties of Kale (Khanyari and GM Dari) is presented in Table 4.10. The data indicates that the leaf phosphorus content is markedly influenced by foliar spray of nano-urea. There is a significant difference in leaf phosphorus content due to the nano-urea treatments of varied concentrations. However, maximum value (0.56% in Khanyari and 0.48% in GM Dari) is observed for the treatment T_4 where the concentration of nano-urea is 2000 ppm which is followed by T_3 treatment (0.52% in Khanyari and 0.45% in G M Dari) where the concentration of urea spray is 2000 ppm . The lowest value is observed for control i.e, no urea application (0.12% in Khanyari and 0.11% in GM Dari). As compared to the soil application of the recommended dose of urea(T_1), there is 14.2% increase in leaf phosphorus content in Khanyari and 15.7% in G M Dari respectively using the foliar spray of 125 ppm concentration of nano-urea.

Table 4.10: Effect of nano-urea on leaf nitrogen content (%) and leaf phosphorus content (%) in Kale

Treatments	Leaf nitrogen content (%)			Leaf phosphorus content (%)		
	Khanyari	**GM Dari**	**Mean**	**Khanyari**	**GM Dari**	**Mean**
Recommended soil application (T_1)	1.56	1.21	1.38	0.18	0.16	0.17
No urea or control (T_2)	1.06	1.03	1.05	0.12	0.11	0.12
urea spray 2000 ppm (T_3)	2.91	1.93	2.44	0.52	0.45	0.47
nano-urea spray 2000 ppm (T_4)	2.97	2.11	2.54	0.56	0.48	0.52
nano-urea spray 1000 ppm (T_5)	2.73	1.90	2.33	0.42	0.41	0.42
nano-urea spray 500 ppm (T_6)	2.51	1.74	2.13	0.33	0.31	0.32
nano-urea spray 250 ppm (T_7)	2.26	1.52	1.89	0.23	0.22	0.23
nano-urea spray 125 ppm (T_8)	1.94	1.41	1.68	0.21	0.19	0.20
Mean	2.24	1.60		0.32	0.29	
	C.D ($p \leq 0.05$) Variety: **0.44** Treatment: **0.99** Variety×Treatment: **1.43**			**C.D ($p \leq 0.05$)** Variety: **0.008** Treatment: **0.04** Variety×Treatment: **0.048**		

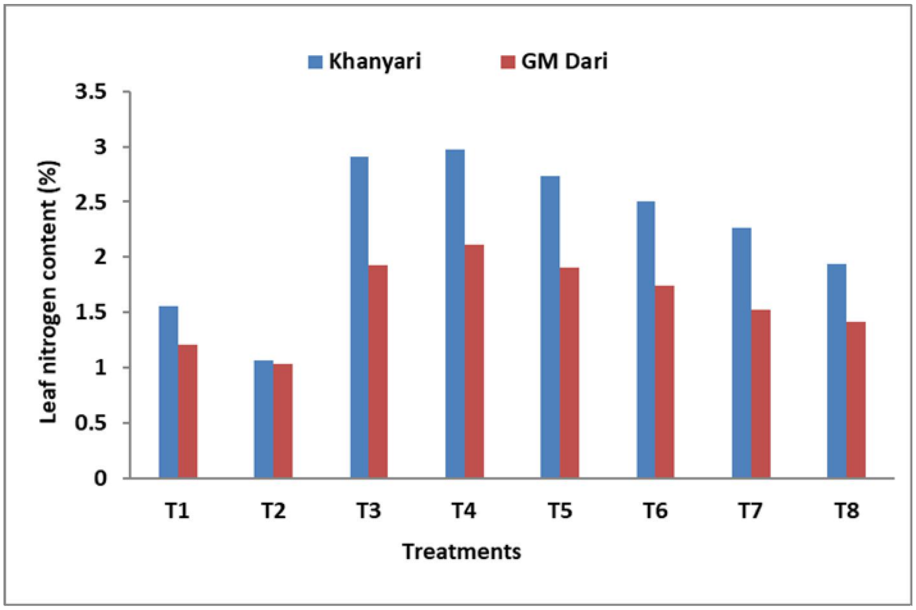


Fig.18: Graphical representation of effect of nano-urea on leaf nitrogen content in Kale

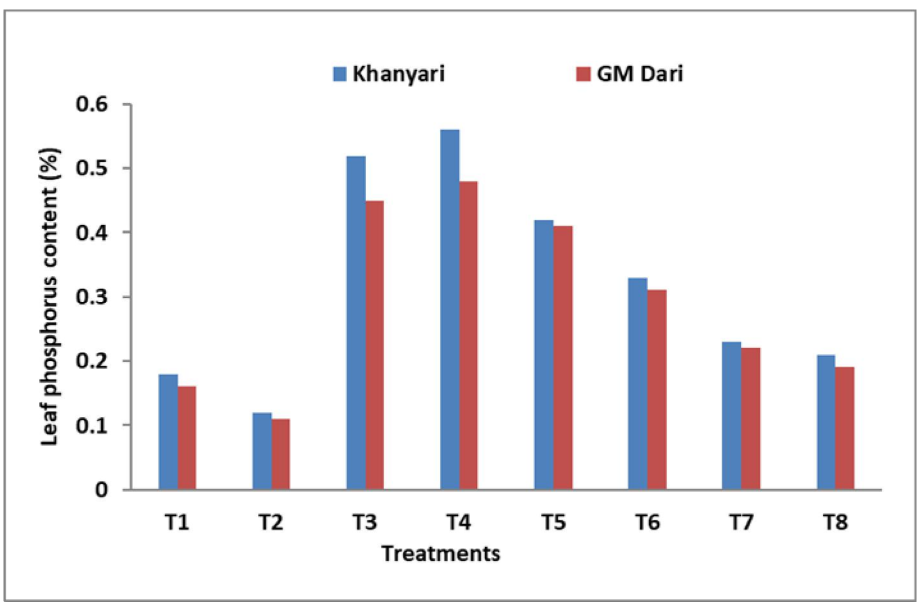


Fig.19: Graphical representation of effect of nano-urea on leaf phosphorus content in Kale

4.3.5 Effect of nano-urea on leaf potassium content (%)

The data in Table 4.11 indicates that the leaf potassium content in both the varieties of Kale (Khanyari and GM Dari) is noticeably affected by the different concentrations of nano-urea. There is a significant difference in leaf potassium content due to the nano-urea treatments of varied concentrations. However, maximum value (2.43% in Khanyari and 1.41% in GM Dari) is observed for the treatment T_4 where the concentration of nano-urea is 2000 ppm which is followed by T_3 treatment (2.39% in Khanyari and 1.36% in G M Dari) where the concentration of urea spray is 2000 ppm. The lowest value is observed for control i.e, no urea application (0.81% in Khanyari and 0.73% in GM Dari). As compared to the soil application of the recommended dose of urea(T_1), there is 11.1% increase in leaf potassium content in Khanyari and 3.5% in G M Dari respectively using foliar spray of 125 ppm concentration of nano-urea.

4.3.6 Effect of nano-urea on leaf calcium content (%)

The perusal of data (Table 4.11) reveals that the leaf calcium content in both the varieties of Kale (Khanyari and GM Dari) is distinctly influenced by the varied concentrations of nano-urea. There is a significant difference in leaf calcium content due to the nano-urea treatments of varied concentrations. However, maximum value (0.070% in Khanyari and 0.066% in GM Dari) is observed for the treatment T_4 where the concentration of nano-urea is 2000 ppm which is followed by T_3 treatment (0.067% in Khanyari and 0.062% in G M Dari) where the concentration of urea spray is 2000 ppm. The lowest value is observed for control i.e, no urea application (0.038% in Khanyari and 0.036% in GM Dari).). As compared to the soil application of the recommended dose of urea(T_1), there is 14.8% increase in leaf calcium content in Khanyari and 14.2% in G M Dari respectively using foliar spray of 125 ppm of nano-urea.

Table 4.11: Effect of nano-urea on leaf potassium content (%) and leaf calcium content (%) in Kale

Treatments	Leaf potassium content (%)			Leaf calcium content (%)		
	Khanyari	**GM Dari**	**Mean**	**Khanyari**	**GM Dari**	**Mean**
Recommended soil application (T_1)	1.85	1.09	1.47	0.046	0.042	0.046
No urea or control (T_2)	0.81	0.73	0.77	0.038	0.036	0.037
urea spray 2000 ppm (T_3)	2.39	1.36	1.87	0.067	0.062	0.065
nano-urea spray 2000 ppm (T_4)	2.43	1.41	1.92	0.070	0.066	0.068
nano-urea spray 1000 ppm (T_5)	2.31	1.31	1.81	0.064	0.060	0.062
nano-urea spray 500 ppm (T_6)	2.21	1.23	1.72	0.058	0.055	0.057
nano-urea spray 250 ppm (T_7)	2.12	1.16	1.64	0.054	0.050	0.053
nano-urea spray 125 ppm (T_8)	2.08	1.13	1.61	0.053	0.049	0.052
Mean	2.02	1.17		0.056	0.052	
	C.D ($p \leq 0.05$) Variety**: 0.29** Treatment: **0.64** Variety×Treatment: **0.93**			**C.D ($p \leq 0.05$)** Variety: **0.002** Treatment: **0.009** Variety×Treatment: **0.011**		

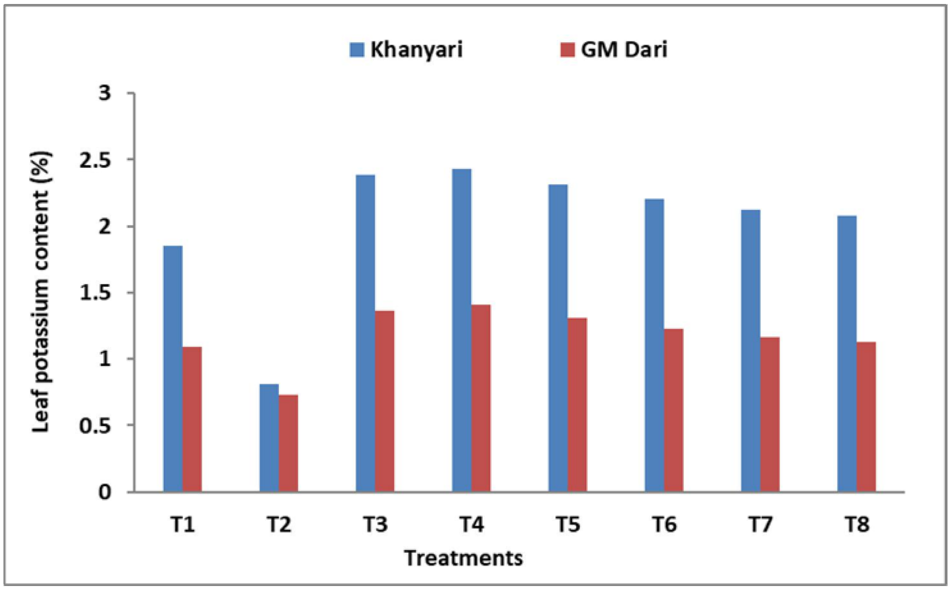


Fig.20: Graphical representation of effect of nano-urea on leaf potassium content in Kale

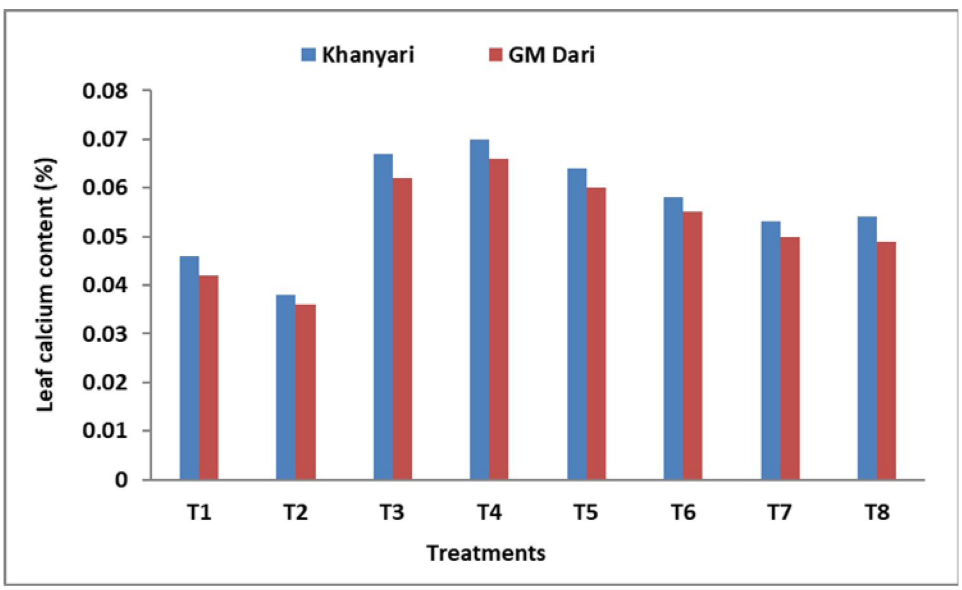


Fig.21: Graphical representation of effect of nano-urea on leaf calcium content in Kale

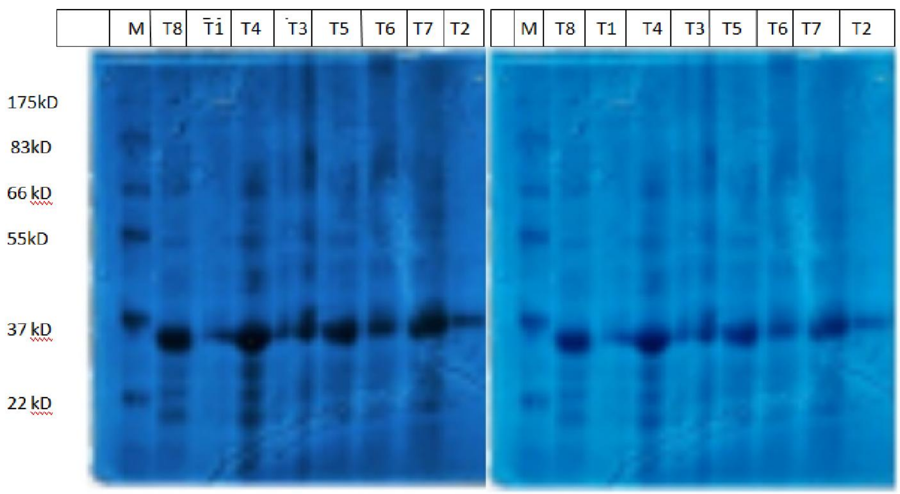
4.3.7 Effect of nano-urea on leaf nitrate content (mg/100g)

The information in Table 4.12 shows that the various nano-urea concentrations have a discernible impact on the leaf nitrate content in both the Khanyari and GM Dari kinds of Kale. The treatments with different doses of nano-urea caused a noticeable variation in the amount of nitrate in the leaves. There is a significant increase in leaf nitrate content by increasing the concentration of nano-urea for both the varieties. However, maximum value (165.30g/100g in Khanyari and 172.20g/100g in GM Dari) is observed for the treatment T_4 where the concentration of nano-urea is 2000 ppm which is followed by T_3 treatment (159.26g/100g in Khanyari and 168.31 g/100g in G M Dari) where the concentration of urea spray is 2000 ppm. As compared to the soil application of the recommended dose of urea (T_1), there is 6.3% increase in leaf nitrate content in Khanyari and 5.8% in G M Dari respectively using foliar spray of 125 ppm concentration of nano-urea. The lowest value is observed for control i.e, no urea application (88.20g/100g in Khanyari and 96.30g/100g in GM Dari). There is a slight increase in the accumulation of nitrate in the leaves where 125 ppm concentration of nano-urea was applied (T_8) in comparison to the soil application of the recommended dose of urea (T_1). But among all nano-urea treatments, T_8 (i.e, 125 ppm) showed the least nitrate accumulation when compared to the use of higher doses of nano-urea.

4.4 Molecular parameter

4.4.1 Protein profiling of leaf samples by SDS page on application of different nano-urea treatments

As shown in the figure, amongst all treatments in both the varieties, the maximum number of protein bands are recorded in T_4 i.e 15 bands in both the varieties of Kale, however in G M Dari the intensity of certain bands is low as compared to those in Khanyari. Band No.2 corresponding to 175kD is present only in T_8 in both the varieties but with low intensity. Band No.3 is present in T_1, T_4,T_3 and T_5 in both the varieties, however with different intensities. Band No. 4



Khanyari **G M Dari**

Fig.24 : Electrophorogram of leaf protein profile of both the varieties of Kale using SDS-PAGE

corresponding to 83 kD is present in T_8 in both the varieties but with less intensity. Band No. 5 corresponding to 66 kD is common in T_8, T_4, T_3, T_6 and T_7 in Khanyari but in G M Dari this band is missing in T_6 and T_7. However in both these varieties, this band is present with higher intensity in T_4. Band No.6 is present in T_4, T_5 and T_7 in both the varieties, however with different intensities. Band No.7 corresponding to 55kD is present in all the treatments in both the varieties but with higher intensity in T_4. Band No.8 is present in T_3, T_4, T_5, T_6 and T_7 with different intensities in both the varieties. Band No. 9 corresponding to 37kD is present in all the treatments of both the varieties and with a higher intensity. Band No.10 is present in T_4, T_5, T_7 and T_8 in Khanyari with different intensities however, this band is absent in T_7 in G M Dari. Band No.11 is present only in T_3 and T_4 in both the varieties. Band No. 12 corresponding to 22kD is present in all the treatments in both the varieties but with different intensities. Band No. 13 is present in all the treatments with different intensities in both the varieties, however its higher intensity is seen in T_4. Band No. 14 and 15 are present only in T_3 and T_4 but with a higher intensity in T_4 in both the varieties.

4.5 Quality parameters

4.5.1 Effect of nano-urea on vitamin C content (mg/100g)

The data in Table 4.12 specifies that the vitamin C content in both the varieties of Kale (Khanyari and GM Dari) is markedly influenced by foliar spray of nano-urea. There is a significant difference in vitamin C content due to the nano-urea treatments of varied concentrations. However, maximum value (150.20g/100g in Khanyari and 148.30 g/100g in GM Dari) is observed for the treatment T_8 where the concentration of nano-urea is 125 ppm which is followed by T_7 treatment (136.20 g/100g in Khanyari and 134.20 g/100g in G M Dari) where the concentration of nano-urea is 250 ppm. The lowest value is observed for control i.e, no urea application (67.50g/100g in Khanyari and 64.50g/100g in GM Dari). As compared to the soil application of recommended dose of urea (T_1), there is 16.7% increase in vitamin C content in Khanyari and 18.3% in G M Dari respectively using foliar spray of 125 ppm concentration of nano-urea.

Table 4.12: Effect of nano-urea on leaf nitrate content (mg/100g) and vitamin C content (mg/100g) in Kale

Treatments	Leaf nitrate content (mg/100g)			Vitamin C content (mg/100g)		
	Khanyari	GM Dari	Mean	Khanyari	GM Dari	Mean
Recommended soil application (T_1)	120.10	128.50	125.80	125.10	121.20	123.15
No urea or control (T_2)	88.20	96.30	92.25	67.50	64.50	66.00
urea spray 2000 ppm (T_3)	159.26	168.31	163.20	88.20	85.20	86.70
nano-urea spray 2000 ppm (T_4)	165.30	172.20	168.75	93.60	91.20	92.40
nano-urea spray 1000 ppm (T_5)	156.10	163.20	159.65	107.20	105.20	106.70
nano-urea spray 500 ppm (T_6)	148.30	151.10	149.70	122.30	120.20	121.75
nano-urea spray 250 ppm (T_7)	136.20	141.20	138.70	136.20	134.20	135.20
nano-urea spray 125 ppm (T_8)	128.30	136.50	132.40	150.20	148.30	149.25
Mean	132.73	139.89		111.28	108.75	
	C.D ($p \leq 0.05$) Variety: **5.98** Treatment: **7.66** Variety×Treatment: **13.64**			**C.D ($p \leq 0.05$)** Variety: **1.20** Treatment: **1.98** Variety×Treatment: **3.18**		

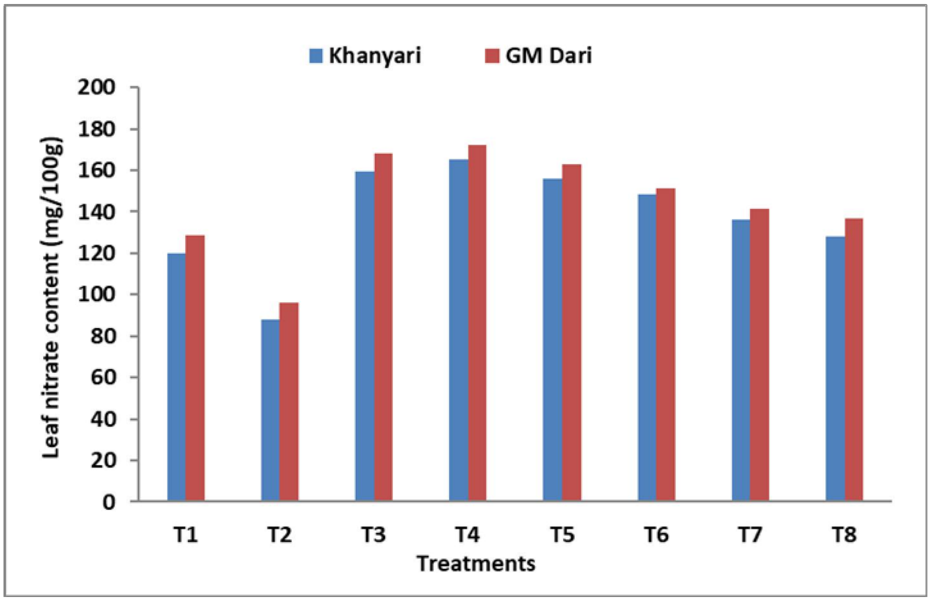


Fig.22: Graphical representation of effect of nano-urea on leaf nitrate content in Kale

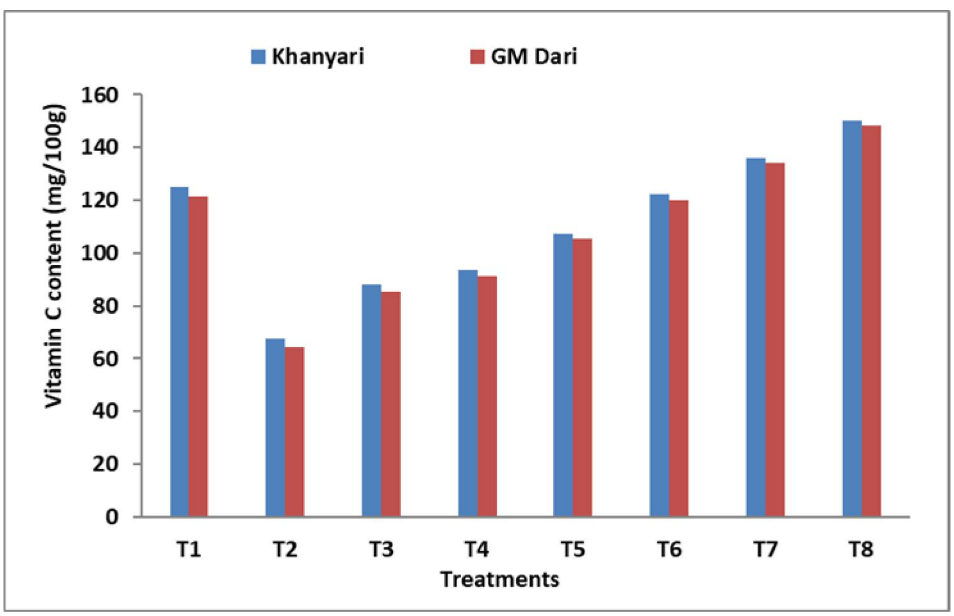


Fig.23: Graphical representation of effect of nano-urea on Vitamin C content in Kale

4.5.2 Effect of nano-urea on total antioxidant activity (mg GAE g^{-1})

The data on total antioxidant activity in both the varieties of Kale (Khanyari and GM Dari) is presented in Table 4.13. The data indicates that the total antioxidant activity is markedly influenced by foliar spray of nano-urea. There is a significant difference in total antioxidant activity due to the nano-urea treatments of varied concentrations. However, maximum value (56.30 mg GAE g^{-1} in Khanyari and 45.60 mg GAE g^{-1} in GM Dari) is observed for the treatment T_8 where the concentration of nano-urea is 125 ppm which is followed by T_7 treatment (51.30 mg GAE g^{-1} in Khanyari and 41.30 mg GAE g^{-1} in GM Dari) where the concentration of nano-urea is (250 ppm). The lowest value is observed for control i.e, no urea application (31.30 mg GAE g^{-1} in Khanyari and 22.60 mg GAE g^{-1} in GM Dari) . As compared to the soil application of the recommended dose of urea(T_1), there is 14.3% increase in total antioxidant activity in Khanyari and 14.2% in G M Dari respectively using foliar spray of 125 ppm concentration of nano-urea.

4.5.3 Effect of nano-urea on protein content (g/100g)

The perusal of data (Table 4.13) reveals that the crude protein content is markedly influenced by foliar spray of nano-urea. There is a significant increase in crude protein content with increase in the concentrations of the nano-urea liquid in the spray solutions. However, maximum value (18.61g/100g in Khanyari and 13.24 g/100g in GM Dari) is observed for the treatment T_4 where the concentration of nano-urea is 2000 ppm which is followed by T_3 treatment (18.18 g/100 g in Khanyari and 13.05 g/100g in G M Dari) where the concentration of urea spray is 2000 ppm. The lowest value is observed for control i.e, no urea application (6.61g/100g in Khanyari and 6.42g/100g in GM Dari). As compared to the soil application of the recommended dose of urea(T_1), there is 15.8% increase in crude protein content in Khanyari and 13.7% in G M Dari respectively using foliar spray of 125 ppm concentration of nano-urea.

Table 4.13: Effect of nano-urea on total antioxidant activity (mg GAE g^{-1}) and crude protein content (g/100g) in Kale

Treatments	Total antioxidant activity (mg GAE g^{-1})			Crude protein content (g/100g)		
	Khanyari	GM Dari	Mean	Khanyari	GM Dari	Mean
Recommended soil application (T_1)	48.20	39.10	43.65	10.20	8.50	9.35
No urea or control (T_2)	31.30	22.60	26.95	6.61	6.42	6.52
urea spray 2000 ppm (T_3)	35.40	26.30	30.85	18.18	13.05	15.34
nano-urea spray 2000 ppm (T_4)	37.50	28.10	32.80	18.61	13.24	15.93
nano-urea spray 1000 ppm (T_5)	44.10	33.10	38.60	17.12	12.11	14.62
nano-urea spray 500 ppm (T_6)	47.20	37.10	42.15	15.74	10.92	13.33
nano-urea spray 250 ppm (T_7)	51.30	41.30	46.30	14.17	9.55	11.86
nano-urea spray 125 ppm (T_8)	56.30	45.60	50.95	12.12	9.86	10.49
Mean	43.91	34.15		14.09	10.45	
	C.D ($p \leq 0.05$) Variety: **3.97** Treatment: **4.62** Variety×Treatment: **8.59**			**C.D ($p \leq 0.05$)** Variety: **1.12** Treatment: **1.50** Variety×Treatment: **2.62**		

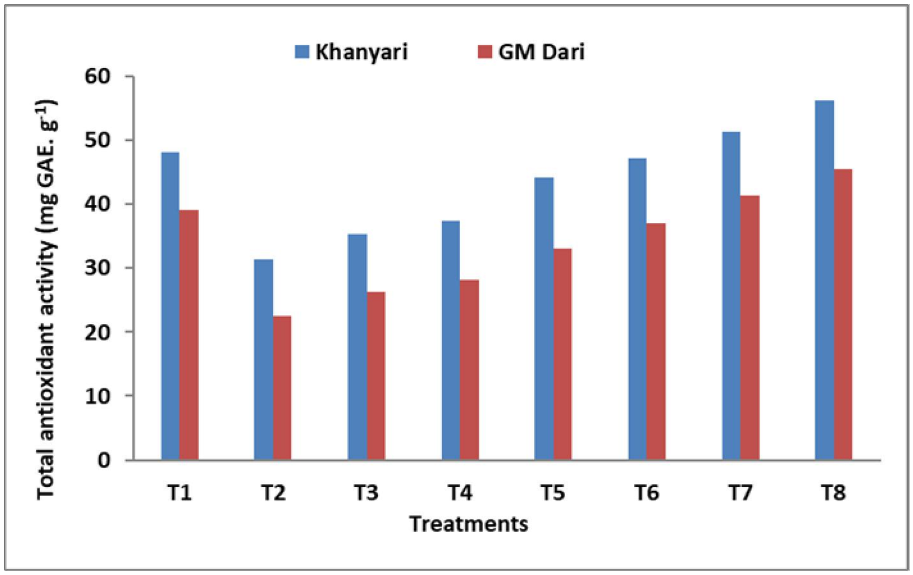


Fig.25: Graphical representation of effect of nano-urea on total antioxidant activity in Kale

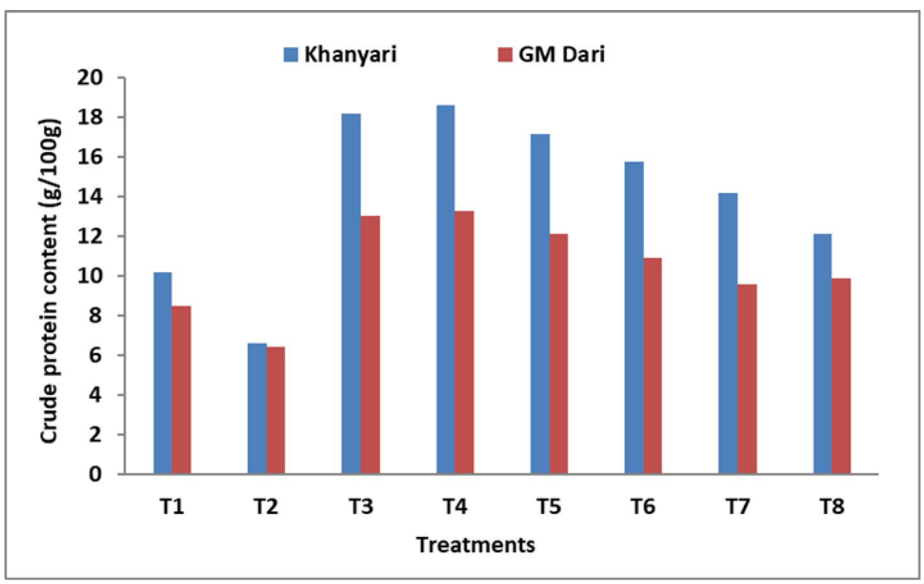


Fig.26: Graphical representation of effect of nano-urea on crude protein content in Kale

4.6 Effect of nano-urea on leaf color

The data on leaf color in both the varieties of Kale (Khanyari and GM Dari) is presented in table 4.14. The color was measured by Hunter lab and these values were significantly influenced by different nano-urea treatments. All color values; L*, a*, b*, Hue angle (°) and chroma(C*) of both the varieties of Kale were significantly different in different treatments. The L* value was higher in control (45.27 in Khanyari and 46.21in G M Dari) for both the varieties, while as the lowest L* value (39.07 for Khanyari and 39.14 for G M Dari) was observed for T_4 treatment which means that the leaves receiving T4 treatment (2000 ppm nano-urea) were darker as compared to the the leaves receiving the other treatments of nano-urea. Negative values of a* represent green and the plants receiving T_4 treatment showed higher value which means greener hue compared to all other treatments. Maximum value (-4.71 for Khanyari and -5.95 for G M Dari) for a* was observed for T_4 treatment in both the varieties while as the minimum value (-7.13 for Khanyari and -8.04 for G M Dari) was seen for control. Positive values of b* represent a higher proportion of yellow color over blue. Lower value (10.77 for Khanyari and 12.26 for G M Dari) was observed for treatment T_4 where a higher concentration of nano-urea was used in the spray solution while the maximum value (15.41 in Khanyari and 16.52 in G M Dari) was observed for control in both the varieties. Higher hue angle was observed for T_4 treatment for both the varieties (121.68° for Khanyari and 121.66° for G M Dari) where the value for chroma was (12.76 for Khanyari and 11.32 for G M Dari) respectively while as the lower hue angle (115.74° for Khanyari and 115.70° for G M Dari) was seen in treatment T_2 with value of chroma (18.03 in Khanyari and 16.57 in G M Dari) respectively for both the varieties.

Table 4.14: Effect of nano-urea on leaf color (L* a* b*) in Kale

Treatments	Leaf color (L* a* b*)								
	L			a			b		
	Khanyari	GM Dari	Mean	Khanyari	GM Dari	Mean	Khanyari	GM Dari	Mean
Recommended soil application (T_1)	43.41	43.71	42.89	-6.90	-7.93	-7.41	14.63	15.77	15.09
No urea or control (T_2)	45.27	46.21	44.74	-7.13	-8.04	-7.58	15.41	16.52	15.97
urea spray 2000 ppm (T_3)	39.21	39.25	39.65	-6.13	-7.02	-6.57	11.01	12.26	11.51
nano-urea spray 2000 ppm (T_4)	39.07	39.14	39.05	-4.71	-5.95	-5.33	10.77	12.01	10.02
nano-urea spray 1000 ppm (T_5)	40.01	40.05	40.51	-6.41	-7.53	-6.97	12.62	13.92	13.27
nano-urea spray 500 ppm (T_6)	40.21	40.64	40.90	-6.51	-7.71	-7.11	13.02	14.01	13.51
nano-urea spray 250 ppm (T_7)	40.62	41.41	41.99	-6.62	-7.73	-7.17	14.41	15.52	14.21
nano-urea spray 125 ppm (T_8)	42.21	42.57	42.40	-6.80	-7.80	-7.30	14.51	15.68	14.97
Mean	40.87	41.51		-6.40	-7.46		13.40	14.15	
	C.D ($p \leq 0.05$) Variety**: 0.05** Treatment: **0.06** Variety×Treatment: **0.21**			**C.D ($p \leq 0.05$)** Variety: **0.026** Treatment: **0.052** Variety×Treatment: **0.074**			**C.D ($p \leq 0.05$)** Variety: **0.04** Treatment: **0.06** Variety×Treatment: **0.12**		

Table 4.15: Effect of nano-urea on chroma (C*) and hue angle (°) in Kale

Treatments	Chroma (C*)			Hue angle (°)		
	Khanyari	GM Dari	Mean	Khanyari	GM Dari	Mean
Recommended soil application (T_1)	16.98	15.22	16.10	121.13	121.10	121.11
No urea or control (T_2)	18.03	16.57	17.30	115.74	115.70	115.85
urea spray 2000 ppm (T_3)	13.91	12.58	13.25	121.51	121.48	121.42
nano-urea spray 2000 ppm (T_4)	12.76	11.32	12.04	121.68	121.66	121.71
nano-urea spray 1000 ppm (T_5)	14.14	14.03	14.08	121.47	121.45	121.55
nano-urea spray 500 ppm (T_6)	14.46	14.18	14.32	121.35	121.32	121.30
nano-urea spray 250 ppm (T_7)	15.91	14.71	15.31	121.24	121.21	121.25
nano-urea spray 125 ppm (T_8)	16.71	15.13	15.92	121.20	121.17	121.15
Mean	16.32	14.79		120.66	120.63	
	C.D ($p \leq 0.05$) Variety: **0.06** Treatment: **0.12** Variety×Treatment: **0.18**			**C.D ($p \leq 0.05$)** Variety: **0.01** Treatment: **0.34** Variety×Treatment: **0.35**		

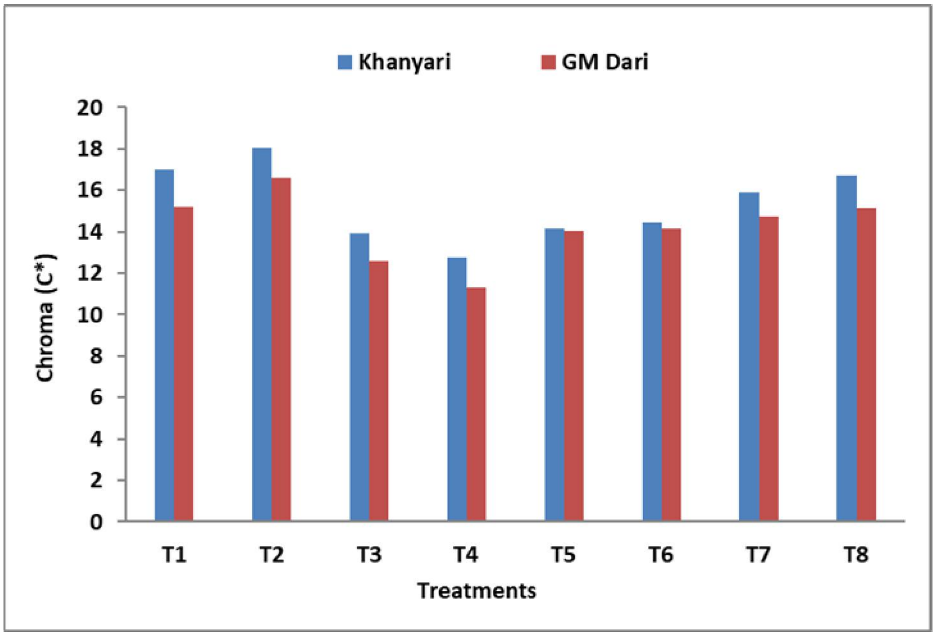


Fig.27: Graphical representation of effect of nano-urea on Chroma in Kale

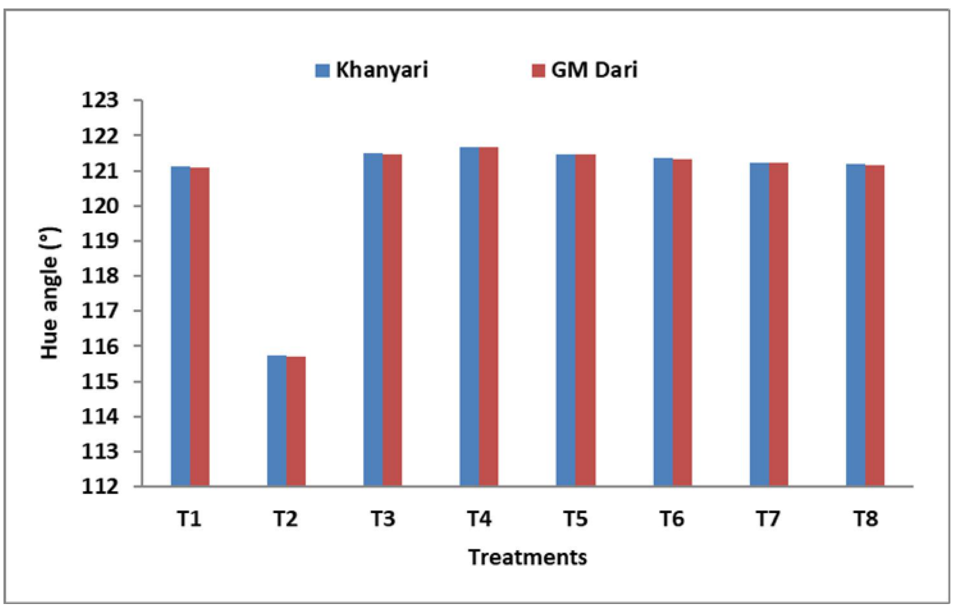


Fig.28: Graphical representation of effect of nano-urea on Hue angle in Kale

4.7 Yield parameters

4.7.1 Effect of nano-urea on yield per plant (g)

The perusal of data (Table 4.16) reveals that the yield per plant in both the varieties of Kale (Khanyari and GM Dari) is markedly influenced by foliar spray of nano-urea. There is a significant difference in the yield per plant due to the Nano-urea treatments of varied concentrations. However, maximum value (104.10g/plant in Khanyari and 85.10g/plant in GM Dari) is observed for the treatment T_8 where the concentration of nano-urea is 125 ppm which is followed by T_7 treatment (82.40g/plant in Khanyari and 72.10 g/plant in GM Dari) where the concentration of nano-urea is 250 ppm . The lowest value is observed for control i.e, no urea application (25.30g/plant in Khanyari and 17.20g/plant in GM Dari). As compared to the soil application of the recommended dose of urea(T_1), there is 24% increase in yield per plant in Khanyari and 21% in GM Dari respectively using foliar spray of 125 ppm concentration of nano-urea.

4.7.2 Effect of nano-urea on yield per hectare (t)

Table 4.16 shows the information on yield per hectare for both the Khanyari and GM Dari cultivars of Kale. The data shows that the application of foliar nano-urea has a significant impact on the yield per hectare. There is a significant difference in yield per hectare due to the nano-urea treatments of varied concentrations. However, maximum value (34.50 t ha^{-1} in Khanyari and 27.10 t ha^{-1} in GM Dari) is observed for the treatment T_8 where the concentration of nano-urea is 125 ppm which is followed by T_7 treatment (31.20 t ha^{-1} in Khanyari and 24.10 t ha^{-1} in GM Dari) where the concentration of nano-urea is (250 ppm) . The lowest value is observed for control i.e, no urea application (11.80 t ha^{-1} in Khanyari and 8.20 t ha^{-1} in GM Dari). As compared to the soil application of the recommended dose of urea(T_1), there is 15.3% increase in yield per hectare in Khanyari and 17.2% in G M Dari respectively using foliar spray of 125 ppm of nano-urea.

T1 (15 DAS)

T1 (At harvesting time)

T8 (15 DAS)

T8(At harvesting time)

Plate 5: Comparison of Recommended soil application of urea (T1) to the best treatment T8 (125 ppm of nano-urea) in Khanyari

T1 (15 DAS)

T1(At harvesting time)

T8 (15 DAS)

T8(At harvesting time)

Plate 6: Comparison of Recommended soil application of urea (T1) to the best treatment T8 (125 ppm of nano-urea) in GM Dari

Table 4.16: Effect of nano-urea on yield per plant (g) and yield per hectare (t) in Kale

Treatments	Yield per plant (g)			Yield per hectare (t)		
	Khanyari	GM Dari	Mean	Khanyari	GM Dari	Mean
Recommended soil application (T_1)	78.20	67.20	72.70	29.20	22.30	25.75
No urea or control (T_2)	25.30	17.20	21.25	11.80	8.20	10.00
urea spray 2000 ppm (T_3)	37.10	29.20	33.15	16.10	10.50	13.30
nano-urea spray 2000 ppm (T_4)	42.50	33.30	37.90	18.10	12.20	15.15
nano-urea spray 1000 ppm (T_5)	51.30	42.10	46.70	21.60	15.20	18.40
nano-urea spray 500 ppm (T_6)	62.30	53.10	57.70	28.10	21.10	24.60
nano-urea spray 250 ppm (T_7)	82.40	72.10	77.25	31.20	24.10	27.65
nano-urea spray 125 ppm (T_8)	104.10	85.10	94.60	34.50	27.10	30.80
Mean	60.40	49.91		23.82	17.58	
	C.D ($p \leq 0.05$) Variety: **2.09** Treatment: **2.74** Variety×Treatment: **4.83**			**C.D ($p \leq 0.05$)** Variety: **3.99** Treatment: **4.28** Variety×Treatment: **8.27**		

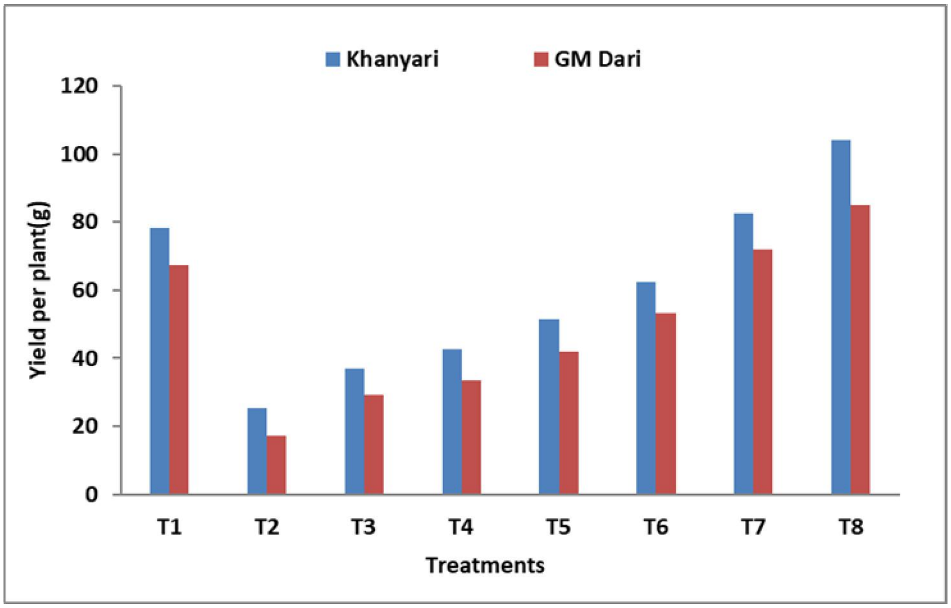


Fig.29: Graphical representation of effect of nano-urea on yield per plant in Kale

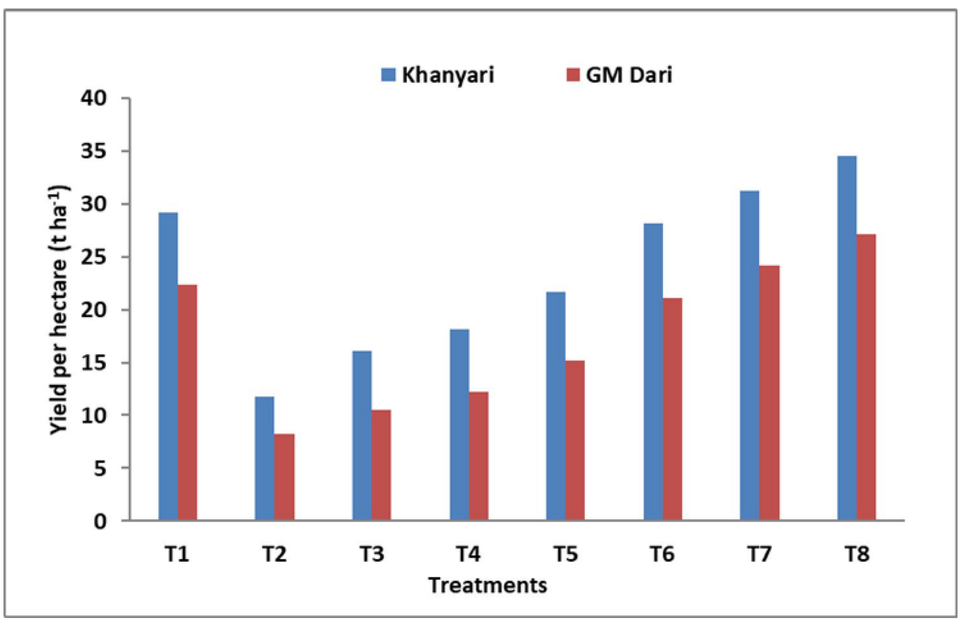


Fig. 30: Graphical representation of effect of nano-urea on yield per hectare in Kale

Since an increase in yield was observed by decreasing the concentration of nano-urea, two more concentrations were tested in order to check their effect on the plant growth. These two concentrations included : 100 ppm of nano-urea (2.5 mL L^{-1}) and 50 ppm (1.25 mL L^{-1}) of nano-urea. The yields obtained were as follows:

Treatments	**Yield (t ha^{-1})**	
	Khanyari	**G M Dari**
nano-urea spray (100 ppm)	14.60	10.80
nano-urea spray (50 ppm)	9.70	7.90

In order to check the effectiveness of nano-urea under field conditions, additional work was done in which a field trail was conducted on 16th April 2022, in which both the varieties of Kale were planted in the field. Same results were observed in the field as were seen in the pot experiment.Seedlings were raised in greenhouse, where the first 1st foliar spray of nano-urea was given to them. After that the seedlings were transplanted to the field 20 DAS and the other two sprays were given to both the varieties in field.

Seedling nursery

Field trial

Foliar application of nano-urea using electric sprayer

Plot receiving the best treatment (125 ppm nano-urea N/L)

Plate 7: Application of different nano-urea treatments in field conditions

Chapter -5

DISCUSSION

From very early times, attempts have been made for increasing production by selecting suitable varieties, adopting improved cultural practices, suitable agrotechniques and plant protection measures. In such an endeavour nanofertilizers have become an important aid in the last decade.

Kale (*Brassica oleracea* var *acephala*) is an important temperate crop in the Kashmir valley. It is available round the year in the hilly regions. In Kashmir valley, kale is grown in all vegetable growing areas during summer and winter, it is also used as culinary and dietic product being rich in minerals, vitamins and proteins.

However, excess use of urea causes environmental issues and results in susceptibility of crops to insects, pests and diseases. Part of urea gets converted in NOx which is emitted to the atmosphere and causes pollution. Runoff of urea to rivers and oceans causes excessive algal growth and excess nitrates leaching to ground/ drinking water are very harmful to human health. In order to reduce such deleterious effects, an approach is approved by FCO, Govt. of India i.e the nano-urea which is a nano-technology based revolutionary agri-input, which provides nitrogen fertilizer to the plants. It is cost effective and compatible with most of the agrochemicals, bio-stimulants and speciality fertilizers for application to crops. Nano-urea will help in improving the soil health, improving aquatic life, reduce nitrate leaching losses and improve ground water quality.

During the present investigation efforts were made to investigate the potential of nano-urea in relation to growth, yield and quality of Kale. Considering the importance of nano fertilizers and their utility, the present investigation is described under the following headings:

5.1 Plant growth parameters

5.2 Physiological parameters

5.3 Biochemical parameters

5.4 Molecular parameters

5.5 Quality parameters

5.6 Leaf color

5.7 Yield parameters

5.1 Plant growth parameters

The different concentrations of nano-urea had a significant influence on the growth components of kale. The data related to the plant growth parameters revealed that lowest concentration of nano-urea used in the spray solution i.e 125 ppm proved to be superior over all other nano-urea treatments with respect to plant height, number of leaves, leaf area, leaf fresh weight and leaf dry weight. This concentration of nano-urea lead to improvement of all these parameters and the higher concentrations lead to decrease in them. Maximum plant height (88.50 cm for Khanyari and 48.20 for G M Dari), number of leaves (13.40 for Khanyari and 11.40 for G M Dari), leaf area (1910.63 cm^2 for Khanyari and 1703.97 cm^2 for G M Dari), fresh weight of leaves (109.20g/plant for Khanyari and 100.20g/plant for G M Dari), and dry weight of leaves(14.70g/plant for Khanyari and 13.20g/plant for G M Dari) was observed at 125 ppm concentration of nano urea. By increasing the concentration of nano-urea, all these parameters decreased gradually. The lowest values were observed for control (40.30cm for Khanyari and 33.30cm for G M Dari) for plant height (5.30 for Khanyari and 4.20 for G M Dari) for number of leaves (551.23cm^2 for Khanyari and 501.07cm^2 for G M Dari) for leaf area (30.30g/plant for Khanyari and 28.10 g/plant for GM Dari) for fresh weight of leaves and (5.20 for Khanyari and 4.30 for G M Dari)for dry weight of leaves respectively. The increase in all the plant growth parameters due to the nano nitrogen application could be due to the reason that nano fertilizers increase the availability of nutrient to the plant thereby increasing more shoot length of the plant. Also, nano fertilizers have high reactivity because of more specific surface area, hence nano particles increase the activity of the water and N , P, K are absorbed by the plants along with water which leads to the increase in overall plant growth. The shoot and root promoted effects of nano particles could also be explained by the fact that nano particles lead to relative gene expression of the cell division genes and water channel genes which ultimately improve plant growth by cell enlargement and cell division. But, the application of higher doses of nano nitrogen fertilizer (nano-urea) leads to reduction in plant growth because of higher accumulation of nitrogen in the leaves which results in the down regulation of photosynthesis due to the accumulation of starch granules in the chloroplasts of the leaves (Bondada *et al.,* 2003). Similar observations were reported by (Heba *et al.,* 2016) in wheat where all plant development indicators increased after the application of Nano chitosan NPK at the lowest concentration (Nano 10).

Unlike macronutrients nanomaterials have particular properties, such as surface effect, volume effect and quantum size effect (Auffan *et al*., 2009). Nanomaterials can enhance crop seed germination and promote plant growth (Zheng *et al.,* 2005 and Lin *et al.,* 2009). By the application of nano-nitrogen fertilizers, nitrogen efficiency of lettuce grown under different irrigation regimes can be enhanced (Mohamed *et al.,* 2022). Growth attributes in plants were increased by the application of nitrogen in nano form because nitrogen use efficiency is increased by fertigation in drip irrigation (Eleiwa *et al*.,2012). The results are also consistent with Moosapoor *et al.* (2013) who examined that foliar application of nano fertilizers play a significant role in photosynthesis, increased leaf area and finally increased yield. Fertilizer use efficiency is significantly enhanced by the application of nutrients in nano form (Schwab *et al.,* 2015). This behavior of nano fertilizers lead to formation of new openings that penetrate the cell wall and stimulate the absorption of water and other essential nutrients that encourage the growth of plant (Abyaneh and Maryam, 2014). Nano fertilizers were more effective and efficient than traditional fertilizers due to their positive effects on growth, quality and nutrition of crops as well as reducing the stress in plants (Morales-Diaz *et al*., 2017, Singh, 2017, Ali and Al-juthery, 2017). The nano form of nutrients make a complex with numerous specific and non specific membrane transporter proteins or chemicals in the root exudates then directly transported to the plants (Shukla *et al.* 2016). The high N-use efficiency of nano fertilizer is supported by the results of Subramanian and Tarafdar (2011) on ^{15}N, indicating that N-use efficiency from nano fertilizer reached 82% compared to 42% from conventional fertilizer (urea) which suggested that nano fertilizers may regulate nutrient release that commensurate with plant requirement. The foliar nitrogen application is a practical and sustainable way and it could reduce nitrogen loss to the environment (Gagne *et al.,* 2019). It is also noted that the nano particles can move in all types of tissues including both stomatal and cuticular pathways and that the washing did not remove significant amount (Larue *et al.,* 2014).

The direct exposure of wheat plants to specific types of nanoparticles cause significant increase in all growth variables determined at optimum concentrations of nanosolution (Mahmoodzadeh *et al.,* 2013). The nanoparticles effected the seed germination mechanism by increasing the water absorption by the seeds; increased nitrate reductase enzyme concentration; promoted seed antioxidant system; reduced antioxidant stress by reducing H_2O_2, superoxide radicals, and malonyldialdehyde content; and increased some enzymes such as superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase and catalase activities, which improved seed germination in some plant species (Lu *et al.,* 2002; Lei *et al.,* 2008 and Feizi *et al.,* 2012). The uptake efficiency and the effect of various nanoparticles on the growth and metabolic functions vary among plants (Nair *et al.,* 2010)

5.2 Physiological parameters

The net photosynthetic rate, stomatal conductance and transpiration rate were found to be maximum in T_8, where the foliar application of nano-urea with concentration 125 ppm was superior to all other treatments. The maximum value being 17.20µmole $m^{-2}sec^{-1}$ in Khanyari and 15.40 µmole $m^{-2}sec^{-1}$ in G M Dari for net photosynthetic rate, 0.51 mmole $m^{-2}sec^{-1}$ in Khanyari and 0.40 mmole $m^{-2}sec^{-1}$ in G M Dari for stomatal conductance and 5.71 µmole $m^{-2}sec^{-1}$ in Khanyari and 4.70 µmole $m^{-2}sec^{-1}$ in G M Dari for transpiration rate respectively. The lowest net photosynthetic rate (11.30 µmole m^{-2} sec^{-1} in Khanyari and 9.20 µmole m^{-2} sec^{-1} in GM Dari), stomatal conductance (0.24 mmole m^{-2} sec^{-1} in Khanyari and 0.22 mmole m^{-2} sec^{-1} in GM Dari) and transpiration rate(3.41 µmole m^{-2} sec^{-1} in Khanyari and 2.40 µmole m^{-2} sec^{-1} in G M Dari) were obtained for control . The decrease in net photosynthetic rate, stomatal conductace and transpiration rate with increasing doses of nano-urea could be ascribed to the N induced starch accumulation with higher rates of nitrogen which is a component in the down regulation of photosynthesis. The inhibition of photosynthetic activity in leaves is due to the change in ultrastucture of their chloroplasts which occurs due to the accumulation of starch granules. Similar observations were reported by Xiaomei Fang *et al*. (2018) in buckwheat where the application of nitrogen fertilizer first increased the net photosynthetic rate, stomatal conductance and transpiration rate and then decreased them with the application of higher rates of nitrogen fertilizer. Similar results were also received by Hongzhi Zhang *et al.* (2021) in wheat by the application of nitrogen fertilizer. Hui Wang *et al.* (2021) while working on Nano-Se reported that Nano-Se foliar application improved the net photosynthetic rate, stomatal conductance of leaves and transpiration rate.

The leaf chlorophyll a, chlorophyll b, total chlorophyll content and leaf carotenoid content were significantly influenced by nano-urea treatments. However the maximum value (1.45 mg/g in Khanyari and 1.28 mg/g in G M Dari) for leaf chlorophyll a content was found in T_4 treatment while as the minimum results (1.02 mg/g in Khanyari and 0.91 mg/g in G M Dari) were obtained for control. The maximum value (0.71 mg/g in Khanyari and 0.56 mg/g in G M Dari) for leaf chlorphyll b content was observed for treatment T_4 while as the lowest results (0.23 mg/g in Khanyari and 0.21 mg/g in G M Dari) were seen in control. Same trend was also followed for total leaf chlorophyll content where the maximum value (2.16 mg/g in Khanyari and 1.85 mg/g in G M Dari) was observed for treatment T_4 while as the lowest value (1.29 mg/g in Khanyari and 1.12 mg/g in G M Dari) was observed in control. The maximum value (0.35 mg/g in Khanyari and 0.33 mg/g in G M Dari) for leaf carotenoid content was obtained for treatment T_4 while as the lowest results (0.21 mg/g in Khanyari and 0.19 mg/g in G M Dari) were obtained for control. The reason for increase in total leaf chlorophyll content with increasing doses of nano nitrogen is that when higher amounts of nitrogen are accumulated in the leaves, most of the nitrogen is partiotened into chlorophyll synthesis and hence its amount increase in the chloroplasts. All these parameters were found to be maximum where the highest concentration of nano-urea i.e 2000 ppm was used for the foliar application to the plant. The present findings corroborate with the findings of EL-Aila *et al.* (2015) in spinach and Merghany *et al.* (2019) in cucumber.

The net assimilation rate and relative growth rate for both the time periods was also found to be maximum in T_8, where the concentration of nano-urea was 125 ppm in the spray solution. The maximum value for the net assimilation rate was 3.71 g m^{-2} day^{-1} for 0-20 days, 4.96 g m^{-2} day^{-1} for 20-40 days in Khanyari and 2.81 g m^{-2} day^{-1} for 0-20 days, 4.11 g m^{-2} day^{-1} for 20-40 days in GM Dari for the treatment T_8 while as the lowest was 1.22 g m^{-2} day^{-1} for 0-20 days, 2.22 g m^{-2} day^{-1} for 20-40 days in Khanyari and 1.13 g m^{-2} $day^{-1}$for 0-20 days, 1.64 g m^{-2} day^{-1} for 20-40 days in GM Dari seen in control. Similarly the maximum value (0.71 g $g^{-1}$$day^{-1}$ for 0-20 days, 0.81 g $g^{-1}$$day^{-1}$ for 20-40 days in Khanyari and 0.62 g $g^{-1}$$day^{-1}$ for 0-20 days, 0.73 g $g^{-1}$$day^{-1}$for 20-40 days in GM Dari) for relative growth rate was received for T_8 while as the lowest value (0.31 g $g^{-1}$$day^{-1}$ for 0-20 days, 0.41 g $g^{-1}day^{-1}$ for 20-40 days in Khanyari and 0.28 g $g^{-1}day^{-1}$ for 0-20 days, 0.32 g $g^{-1}day^{-1}$ for 20-40 days in GM Dari) was observed for control. The reason for increase in dry matter of a plant with application of nano nitrogen fertilizer could be attributed to the fact that the activities of enzymes involved in photosynthetic reactions is increased due to absorption of nano fertilizer and as a result the photosynthetic products increase in the plant leading to increase in the plant biomass. The higher concentrations of Nano urea proved fatal to plant growth and decreased the plant dry matter which led to decrease in the plant growth rate. The present findings corroborate with the findings of Hassnein *et al.* (2019) who worked on nano fertilizers in sugarbeet and reported that the application of nano-nitrogen fertilizer (Sissay) +mineral nitrogen+ mix of nano micro elements (200 ppm) resulted in highest values of relative growth rate and net assimilation rate over 60-90 days from sowing date. Among all the combinations of nitrogen(N) fertilizer ,application of 75% nN through drip irrigation and 25% of nN in foliar application significantly affected the growth and biochemical parameters such as plant biomass, leaf area, absolute growth rate, net assimilation rate (Sharaf-Eldin *et al.*, 2022).

The chlorophyll content increased by increasing the concentration of nano fertilizer. N and K are considered essential mineral in photosynthesis and growth of meristematic tissues (Chandra, 1989). The elevation of chlorophyll content due to the application of nanoparticles is because of the improvement in leaf photosynthesis and decrease in respiration rate (Abdel Wahab *et al.,* 2019). The impact of nano fertilizer on plant growth is due to its role on improving seeds germination which is reflected positively on morphological traits (Subbaiya *et al* 2012). K nano fertilizer and humic acid increase root growth and improve the root system effectiveness that lead to increase the plant height and the plant growth (Ghosh *et al.,* 1981). Also the use of Ferbanat (nano micro humates, aminoacids, natural biological substances, nano micro elements and soil microflora) has increased the growth of cucumber roots, the number of buds and weight of cucumber plant (Ferbanat 2013). Also, the growth of tomato plant was improved with the application of Nanonat and Ferbanat @ 3.0 L /ha (Ekinci *et al.,* 2012). The polymer trapping of urea fertilizers promoted morphological traits of corn plant (Khaveh *et al.,* 2015). Plant cell wall acts as a barrier for easy entry of any external agent including nanoparticles into plant cells. The sieving properties are determined by pore diameter of cell wall ranging from 5 to 50 nm (Fleischer *et al.,* 1999). Hence, only nanoparticles or nanoparticle aggregates with diameter less than the pore diameter of the cell wall could easily pass through and reach the plasma membrane (Moore, 2006; Navarro *et al.,* 2008). There is also a chance for enlargement of pores or induction of new cell wall pores upon interaction with engineered nanoparticles which in turn enhance nanoparticle uptake (Nair *et al.,* 2010). When nanoparticles are applied on leaf surfaces, they enter through the stomatal openings or through the bases of trichomes and then are translocated to various tissues (Uzu *et al.,* 2010). Foliar uptake (uptake through the leaves) of nanoparticles by plants represents another possible way for this purpose. Leaves are important plant organs primarily for photosynthesis, transpiration and gas exchange (Nadakavukaren and McCracken, 1985). Studies on the mechanism of uptake and formation of nanoparticles within plants have also led to more investigations on the use of plants as source for nanoparticle synthesis (Nair *et al.,* 2010).

5.3 Biochemical parameters

The leaf nitrogen, phosphorus, potassium, calcium and nitrate content were significantly influenced by nano-urea treatments. However the maximum value (2.97% in Khanyari and 2.11% in G M Dari) for leaf nitrogen content was found in treatment T_4 while as the minimum results (1.06% in Khanyari and 1.03% in G M Dari) were obtained for control. The maximum value (0.56% in Khanyari and 0.48% in G M Dari) for leaf phosphorus content was observed for treatment T_4 while as the lowest results (0.12% in Khanyari and 0.11% in G M Dari) were seen in control. Same trend was also followed by leaf potassium content where the maximum value (2.43% in Khanyari and 1.41% in G M Dari) was observed for treatment T_4 while as the lowest value(0.81% in Khanyari and 0.73% in G M Dari) was observed in control. The maximum value (0.070 % in Khanyari and 0.066% in G M Dari) for leaf calcium content was obtained for treatment T_4 while as the lowest results(0.038% in Khanyari and 0.036% in G M Dari) were obtained for control. Similarly leaf nitrate content was also highest (165.30 g/100g in Khanyari and 172.20 g/100g in G M Dari) in treatment T_4 where the highest concentration of nano-urea was used and the lowest results (67.50 g/100g in Khanyari and 64.50 g/100g in G M Dari) were obtained for control. All these parameters were found to be maximum where the highest concentration of nano-urea i.e 2000 ppm was used for the foliar application to the plant. The reason for increased uptake of nutrients with the application of nanoparticles is the small size (less than 5 nm) of these nanoparticles which aids their transportation through the cuticular pathway, whereas those with larger sizes travel through the stomatal pathway before arriving to the conducting system, where they aid in the rapid and simple absorption of nutrients by the leaves (Dimpka *et al*., 2015; Qureshi *et al*., 2018). Also, coating of nano and sub nano-composites are capable of regulating the release of nutrients from the fertilizer capsule and nano particles have both positive and negative charged binding site that adsorbs available nitrogen in the soil and curtail different type of losses which results in the increased uptake of nitrogen by the plant. Nanoparticles trigger metabolic activity in the plants which results in increased exudation and acidity. Subsequently, release of phosphate ion occurs as a result of a ligand exchange reaction triggered by plant root exudation, which disrupts the adsorption-desorption equilibrium and releases P in the soil solution where it is easily available for uptake. Additionally, application of nano particles improves carbon balance in crops, which accelerates plant growth leading to increase in the efficiency of micro and macronutrients of plants (Kanoj, Choudhary *et al.,* 2022). Similarly, the increase in nitrate content in the leaves with increasing rates of nano nitrogen is due to the fact that nano fertilizer when foliarly applied to the leaves, stimulates a range of enzymes like nitrase and nitrate reductase which helps the plants to metabolize nitrogen. Similar observations were reported by Yildirim *et al.* (2007) in broccoli, Davarpanah *et al.* (2017) in pomegranate, Merghany *et al.* (2019) in cucumber and Aila *et al.* (2015) in spinach.

The total phenols and total carbohydrates were found to be maximum in T_8 where the concentration of nano-urea used was 125 ppm which was superior to all other treatments. The total phenolic content was found to be maximum in T_8 (2.06 mg GAE. g^{-1} in Khanyari and 1.42 mg GAE. g^{-1} in GM Dari). The lowest results were obtained for control (1.66 mg GAE. g^{-1} in Khanyari and 1.08 mg GAE. g^{-1} in GM Dari). Similarly, total carbohydrates were found to be maximum (5.64 g/100g in Khanyari and 5.06 g/100g in G M Dari) in T_8 while the lowest value (2.09 g/100g in Khanyari and 2.05 g/100 g in G M Dari) was observed for control. The possible reasons for decrease in phenolic content in the leaves with increasing rates of nano nitrogen fertilizer can be attributed to the fact that concentration of proteins and phenols is negatively correlated. Phenylalanine, which is a key substance in synthesis of phenols is preferentially applied into chain protein synthesis rather than phenolic compounds under high nitrogen levels (Elhanafi *et al.,* 2019). With respect to decrease in total carbohydrates with increasing rates of nano nitrogen fertilizer, the reason for this may be the fact that nitrogen is a direct factor which regulates the carbon balance that is the basic element for sugar synthesis and sugar levels decrease with increasing nitrogen. Hence a negative relationship occurs between high doses of nitrogen and carbohydrate accumulation. Nitrogen fertilization increases cell content concentrations (soluble fractions) and changed the sugar composition and bonds established between them in the cell wall. Similar results were obtained by Goncalves Leite *et al.* (2021) in Marandu palisadegrass, Tehranifar *et al.* (2017) in pomegranate, Rose L. Benzo *et al*. (2015) in rice and Nguyen *et al.* (2008) in basil.

The availability of key macronutrients during the growth of the plant has considerable potential to affect phenolic accumulation (Parr and Bolwell, 2000).

Several studies have indicated that the phenolic compounds in grains have effective antioxidant properties, due to the presence of one or more aromatic rings with one or more hydroxyl groups (Zielinski and Kozlowska, 2000). The highest Total phenolic content was obtained by applying HRR-NF which was higher compared to the black and red rice cultivars studied by Ham *et al.* (2013). In addition, the experiment conducted by Edwina *et al.* (2014) on different pigmented landraces of rice showed lower values (233.92-251.38 mg GAE/100g). This implies that the TPC can be enhanced in white rice cultivars through nanofertilizer application and can even exceed the pigmented rice cultivars. Increases in total soluble sugars after N application can be attributed to the important roles of N in chloroplast structure, CO_2 assimilation, and activation of enzymes involved in photosynthesis, which lead to increase in photosynthesis and carbohydrate accumulation and also consequently increase in TSS (Garhwal *et al.,* 2014; Kumar *et al.,* 2014; Stiles, 1999; Ramezanian *et al.,* 2009). Nitrogen fertilization led to increased total, reducing and nonreducing sugars in pomegranate (Prasad and Mali, 2000), and total and reducing sugars in Guava fruits (Sharma *et al.,* 2014). It has been reported that the effect of N fertilizers on sugar content increase may help absorption of other mineral nutrients, improving fruit quality (Sharma *et al.,* 2014).

5.4 Molecular parameter (Protein profiling of leaf samples by SDS page)

As shown in the figure, amongst all treatments in both the varieties, the maximum number of protein bands were recorded in T_4 *i.e* 15 bands in both the varieties of Kale, however in G M Dari the intensity of certain bands was low as compared to those in Khanyari. Band No.2 corresponding to 175kD was present only in T_8 in both the varieties but with low intensity. Band No.3 was present in T_1, T_4, T_3 and T_5 in both the varieties, however with different intensities. Band No. 4 corresponding to 83 kD was present in T_8 in both the varieties but with less intensity. Band No. 5 corresponding to 66 kD was common in T_8, T_4,T_3, T_6 and T_7 in Khanyari but in G M Dari this band was missing in T_6 and T_7. However in both these varieties, this band was present with higher intensity in T_4. Band No.6 was present in T_4, T_5 and T_7 in both the varieties, however with different intensities. Band No.7 corresponding to 55kD was present in all the treatments in both the varieties but with higher intensity in T_4. Band No.8 was present in T_3, T_4, T_5, T_6 and T_7 with different intensities in both the varieties. Band No. 9 corresponding to 37kD was present in all the treatments of both the varieties and with a higher intensity. Band No.10 was present in T_4, T_5, T_7 and T_8 in Khanyari with different intensities however, this band was absent in T_7in G M Dari. Band No.11 was present only in T_3 and T_4 in both the varieties. Band No. 12 corresponding to 22kD was present in all the treatments in both the varieties but with different intensities. Band No. 13 was present in all the treatments with different intensities in both the varieties, however its higher intensity was seen in T_4. Band No. 14 and 15 were present only in T_3 and T_4 but with a higher intensity in T_4 in both the varieties. Similar results were observed by Mawlong *et al.* (2017) in Indian Mustard under recommended dose of nitrogen fertilizer, Landolfi *et al.* (2021) in wheat and Kaur *et al.* (2016) in rice.

5.5 Quality parameters

The quality parameters like vitamin C and total antioxidant activity were significantly affected by the different nano-urea treatments. Maximum vitamin C content (150.20 g/100 g in Khanyari and 148.30 g/100g in G M Dari) was observed for T_8 treatment while the lowest results (67.50 g/100g in Khanyari and 64.50 g/100 g in G M Dari) were observed for control. By increasing the dose of nitrogen fertilizer, the vitamin C content decreased gradually. Same trend was followed by the antioxidant activity which decreased with higher doses of nano-urea. The maximum antioxidant capacity (56.30 mg GAE.g^{-1} in Khanyari and 45.60 mg GAE.g^{-1} in G M Dari) was observed for T_8 treatment while as the lowest value (31.30mg GAE.g^{-1} in Khanyari and 22.60 mg GAE.g^{-1} in G M Dari) was observed in control. The present findings are in accordance with similar type of results obtained by Yildirim *et al.* (2007) in broccoli, Davarpanah *et al.* (2017), in pomegranate, Rose L. Benzo *et al.* (2015) in rice and Nguyen *et al.* (2008) in basil. The possible reasons for decrease in vitamin C content with increasing rates of nano nitrogen could be that higher rates of nitrogen fertilizers increase the concentration of nitrate in the leaves and simultaneously decrease the concentration of ascorbic acid and thus have a negative impact on quality. Also, there is a strong positive correlation between total phenols and antioxidant activity, so when phenolic compounds are reduced, the plant antioxidant activity also decreases. Similar observations were made by Sorensen (1999) and Babik and Elkner (2002) who found that increasing nitrogen application lowered the vitamin C content in broccoli and cabbage. The reducing power of bioactive compounds is generally associated with the presence of reductones (Pin-Der-Duh, 1998), which have been shown to exert antioxidant action by breaking the free radical chains by donating a hydrogen atom (Juntachote and Berghofer, 2005).

The crude protein content was found to be maximum (18.61 g/100g in Khanyari and 13.24 g/100g in G M Dari) for T_4 treatment whereas the lowest value (6.61 g/100g in Khanyari and 6.42 g/100g in G M Dari) was observed in control. By increasing the dose of nano-urea, there was an increase in protein content in the leaves. Khalid (2013) reported same results in anise and coriander and Heba *et al.* (2018) in wheat. These results may be due to the influence of N on the ribosome structure and the biosynthesis of some hormones (gibberellins, auxins, cytokinins) involved in protein synthesis (Jones *et al.,* 1991; El-Wahab and Mohamed, 2007).

5.6 Leaf color

The color was measured by Hunter lab and these values were significantly influenced by different nano-urea treatments. All color values; L*, a*, b*, Hue angle (°) and chroma(C*) of both the varieties of Kale were significantly different for different treatments. The L* value was higher in control (45.27 in Khanyari and 46.21in G M Dari) for both the varieties while as lowest L* value was observed for T_4 treatment (39.07 for Khanyari and 39.14 for G M Dari) which means that the leaves receiving T_4 treatment were darker as compared to the leaves receiving all the other nano-urea treatments. Negative values of a* represent green and the plants receiving T_4 treatment showed higher value which means greener hue compared to all other treatments. Maximum value (-4.71 for Khanyari and -5.95 for G M Dari) for a* was observed for T_4 treatment in both the varieties while as the minimum value (-7.13 for Khanyari and -8.04 for G M Dari) was seen for control. Positive values of b* represent a higher proportion of yellow color over blue. Lower value (10.77 for Khanyari and 12.26 for G M Dari) was observed for treatment T_4 where a higher concentration of nano-urea was used in the spray solution while the maximum value (15.41 in Khanyari and 16.52 in G M Dari) was observed for control in both the varieties. Higher hue angle was observed for T_4 treatment for both the varieties (121.68° for Khanyari and 121.66° for G M Dari) where the value for chroma was (12.76 for Khanyari and 11.32 for G M Dari) respectively while as the lower hue angle (115.74° for Khanyari and 115.70° for G M Dari) was seen in treatment T_2 with value of chroma (18.03 in Khanyari and 16.57 in G M Dari) respectively for both the varieties. Raese *et al.* (2007) also reported similar results in apple by the application of different rates of nitrogen fertilizer. Color was analysed in this experiment by Munsell color chips (Munsell, 1971).

5.7 Yield parameters

The yield per plant and yield per hectare both were influenced by nano-urea treatments significantly. The maximum yield per plant (104.10 g/plant in Khanyari and 85.10 g/plant in G M Dari) was obtained for T_8 whereas the lowest value (25.30 g/plant in Khanyari and 17.20 g/plant in G M Dari) was observed for control. Likewise, yield per hectare was found to be maximum in T_8 (34.50 t ha^{-1} in Khanyari and 27.10 t ha^{-1} in G M Dari) while as the yield per hectare was found to be minimum (11.80 t ha^{-1} in Khanyari and 8.20 t ha^{-1} in G M Dari) for control in both the varieties. Similar results were obtained by Heba *et al.* (2016) in wheat where Nano chitosan fertilizer was used to enhance growth and productivity in wheat. The possible reasons for increased yield are: (i) nanoNPK promotes the plant to absorb the water of soil and nutrients, then the photosynthesis is improved (Wu, 2013); (ii) nano-NPK is considered the biological pump for the plants to absorb nutrients and water (Ma *et al.,* 2009). The activity of water after adding nano-materials was increased and N, P and K were absorbed by the plants along with the absorbed water, thus the production was also increased (Liu and Liao, 2008).

These results are also in agreement with Ferbanat (2013) who found that Ferbanat application increased yield of cabbages with 38–42% and in potatoes with 35–40% compared to control. Similar results also highlighted the importance of the nanofertilizers (Fe, P and K) on improving saffron yield (Amirna *et al.,* 2014). The high concentration of nano fertilizer led to increase in yield of corn (Khaveh *et al.,* 2015). Also, the treatment of seeds by HA (Urea modified hydroxyapatite particle) led to the increase of yield in rice (Subbaiya *et al.,* 2012). Previous studies reported that nano-preparation coated nitrogen fertilizer increased the yield of rice (Wang *et al.,* 2011). The increase in yield is due to increased morphological traits and chlorophyll content. The treatments of nano-nitrogen chelate (NNC), sulfur-coated nano-nitrogen chelate (SNNC), sulfur-coated urea (SCU) fertilizers led to increased potato yield by 56.10%, 59.61%, and 49.76% respectively compared to Urea fertilizer application (Zareabyaneh *et al.,* 2015). This is because these treatments led to reduced nitrate leaching levels by 35.72%, 41.56%, and 9.94% compared to U fertilizer application. Increases in yield and number of fruits per tree with N fertilization have been previously reported in citrus (El-Otmani *et al.,* 2002; Lovatt, 1999), apple (Amiri *et al.,* 2008), sweet cherry (Mitre *et al.,* 2012), and mango (Sarker and Rahim, 2013). Processing of N uptake by plant roots can be limited by soil low temperature and low activity of roots, whereas foliar N application is insensitive to those factors (Etehadnejad and Aboutalebi, 2014). The increase found in fruit set, number of fruits per tree and crop yield with foliar N fertilization can be attributed to the physiological and metabolic roles of N in flowering and fruit set, including supplying carbohydrates, which are necessary for flower bud growth, flower initiation and development, ovule lifespan, effective pollination, and fertility (Etehadnejad and Aboutalebi, 2014; Lovatt, 1994; Stiles, 1999). The significant effects on the yield of fresh seeds, yield of dry seeds, the number of seeds/bush, the number of green pods, the number of mature pods, the number of pods/ bush, the yield of pod, total biomass, harvest index and the weight of 100 seeds of peanut plants was seen when treated with Bohr nanofertilizer (Moosapoor *et al.,* 2013).

Chapter- 6

SUMMARY AND CONCLUSION

The present investigation entitled **"Impact of Nano-urea on Morpho-physiological, Biochemical and Yield Parameters of Kale (*Brassica oleracea* var. *acephala*)"** was a pot experiment carried out during 2021-2022 in the laboratory of Division of Basic Sciences and Humanities, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar. The experiment consisted of 8 treatments, the first treatment (T_1) being the recommended dose of urea, T_2: No urea (water spray only), T_3: urea spray (2000 ppm), T_4: nano-urea spray (2000 ppm), T_5: nano-urea spray (1000 ppm), T_6: nano-urea spray (500 ppm), T_7: nano-urea spray (250 ppm) and T_8: nano-urea spray (125 ppm). These factors were tested in a completely randomized design with three replications per treatment. Observations on plant growth parameters, physiological parameters, biochemical parameters, quality parameters, molecular parameters, quality parameters and yield parameters were recorded. The results obtained were statiscally analyzed and are summarized as under:

6.1 Effect of nano-urea on plant growth parameters

- All the plant growth parameters i.e, plant height 88.50 cm for Khanyari and 48.20 for G M Dari, number of leaves 13.40 for Khanyari and 11.40 for G M Dari , leaf area 1910.63 cm^2 for Khanyari and 1703.97 cm^2 for G M Dari, fresh weight of leaves109.20g/plant for Khanyari and 100.20g/plant for G M Dari, and dry weight of leaves 14.70g/plant for Khanyari and 13.20g/plant for G M Dari for both the varieties were found to be maximum in T_8 treatment where the concentration of nano-urea used was 125 ppm.
- The lowest values for each of these parameters were recorded in control. (40.30cm for Khanyari and 33.30cm for G M Dari) for plant height (5.30 for Khanyari and 4.20 for G M Dari) for number of leaves (551.23cm^2 for

Khanyari and 501.07cm^2 for G M Dari) for leaf area (30.30g/plant for Khanyari and 28.10 g/plant for GM Dari) for fresh weight of leaves and (5.20 for Khanyari and 4.30 for G M Dari) for dry weight of leaves respectively.

6.2 Effect of nano-urea on physiological parameters

- The physiological parameters like net photosynthetic rate, stomatal conductance and transpiration rate were found to be maximum for T8 treatment 17.20µmole m^{-2}sec^{-1} in Khanyari and 15.40 µmole m^{-2}sec^{-1} in G M Dari for net photosynthetic rate, 0.51 mmole m^{-2}sec^{-1} in Khanyari and 0.40 mmole m^{-2}sec^{-1} in G M Dari for stomatal conductance and 5.71 µmole m^{-2}sec^{-1} in Khanyari and 4.70 µmole m^{-2}sec^{-1} in G M Dari for transpiration rate respectively, where 125 ppm nano-urea was used in the spray solution. The lowest values for net photosynthetic rate (11.30 µmole m^{-2} sec^{-1} in Khanyari and 9.20 µmole m^{-2} sec^{-1} in GM Dari), stomatal conductance (0.24 mmole m^{-2} sec^{-1} in Khanyari and 0.22 mmole m^{-2} sec^{-1} in GM Dari) and transpiration rate (3.41 µmole m^{-2} sec^{-1} in Khanyari and 2.40 µmole m^{-2} sec^{-1} in G M Dari) were obtained for control.
- On the other hand, maximum value for leaf chlorophyll a, b, total leaf chlorophyll content and leaf carotenoid content was found for T_4 treatment. Highest value (1.45 mg/g in Khanyari and 1.28 mg/g in G M Dari) for leaf chlorophyll a content was found in treatment T_4 while as the minimum results (1.02 mg/g in Khanyari and 0.91 mg/g in G M Dari) were obtained for control. The maximum value (0.71 mg/g in Khanyari and 0.56 mg/g in G M Dari) for leaf chlorphyll b content was observed for T_4 treatment while as the minimum value (0.23 mg/g in Khanyari and 0.21 mg/g in G M Dari) was seen in control.
- Same trend was also followed by total leaf chlorophyll content where the maximum value (2.16 mg/g in Khanyari and 1.85 mg/g in G M Dari) was

observed for treatment T_4 while as the lowest value(1.29 mg/g in Khanyari and 1.12 mg/g in G M Dari) was observed in control. The maximum value (0.35 mg/g in Khanyari and 0.33 mg/g in G M Dari) for leaf carotenoid content was obtained for treatment T_4 while as the lowest value (0.21 mg/g in Khanyari and 0.19 mg/g in G M Dari) was obtained for control.

- The net assimilation rate and relative growth rate for both the time periods was also found to be maximum in T_8, where the concentration of nano-urea was 125 ppm in the spray solution. The maximum value for the net assimilation rate was 3.71 g m^{-2} day^{-1} for 0-20 days, 4.96 g m^{-2} day^{-1} for 20-40 days in Khanyari and 2.81 g m^{-2} day^{-1} for 0-20 days, 4.11 g m^{-2} day^{-1} for 20-40 days in GM Dari for the treatment T_8 while as the lowest 1.22 g m^{-2} day^{-1} for 0-20 days, 2.22 g m^{-2} day^{-1} for 20-40 days in Khanyari and 1.13 g m^{-2} $day^{-1}$for 0-20 days, 1.64 g m^{-2} day^{-1} for 20-40 days in GM Dari) was seen in control.
- Similarly the maximum value (0.71 g $g^{-1}day^{-1}$ for 0-20 days, 0.81 g $g^{-1}day^{-1}$ for 20-40 days in Khanyari and 0.62 g $g^{-1}day^{-1}$ for 0-20 days, 0.73 g $g^{-1}day^{-1}$for 20-40 days in GM Dari) for relative growth rate was seen for T_8 while as the lowest value (0.31 g $g^{-1}day^{-1}$ for 0-20 days, 0.41 g $g^{-1}day^{-1}$ for 20-40 days in Khanyari and 0.28 g $g^{-1}day^{-1}$ for 0-20 days, 0.32 g $g^{-1}day^{-1}$ for 20-40 days in GM Dari) was observed for control.

6.3 Effect of nano-urea on biochemical parameters

- The total phenolic content was found to be maximum in T_8 (2.06 mg GAE g^{-1} in Khanyari and 1.42 mg GAE g^{-1} in GM Dari). The lowest results were obtained for control (1.66 mg GAE g^{-1} in Khanyari and 1.08 mg GAE g^{-1} in GM Dari).
- Similarly, total carbohydrates were found to be maximum (5.64 g/100g in Khanyari and 5.06 g/100g in G M Dari) in T_8 while the lowest value (2.09

g/100g in Khanyari and 2.05 g/100 g in G M Dari) was observed for control.

- The leaf nitrogen, phosphorus, potassium, calcium and leaf nitrate content was found to be maximum in T_4, where the highest concentration of nano-urea was used. The lowest values for all these parameters were recorded in control. The maximum value(2.97% in Khanyari and 2.11% in G M Dari) for leaf nitrogen content was found in T_4 treatment while as the minimum results (1.06% in Khanyari and 1.03% in G M Dari) were obtained for control.

- The maximum value (0.56% in Khanyari and 0.48% in G M Dari) for leaf phosphorus content was observed for treatment T_4 while as the lowest results (0.12% in Khanyari and 0.11% in G M Dari) were seen in control.

- Same trend was also followed by leaf potassium content where the maximum value (2.43% in Khanyari and 1.41% in G M Dari) was observed for treatment T_4 while as the lowest value (0.81% in Khanyari and 0.73% in G M Dari) was observed in control. The maximum value (0.070 % in Khanyari and 0.066% in G M Dari) for leaf calcium content was obtained for treatment T_4 while as the lowest results(0.038% in Khanyari and 0.036% in G M Dari) were obtained for control.

- Similarly leaf nitrate content was also highest (165.30 g/100g in Khanyari and 172.20 g/100g in G M Dari) in treatment T_4 where the highest concentration of nano-urea was used and the lowest results (67.50 g/100g in Khanyari and 64.50 g/100g in G M Dari) were obtained for control treatment

6.4 Effect of nano-urea on molecular parameter

- Amongst all treatments in both the varieties, the maximum number of protein bands were recorded in T_4 i.e 15 bands in both the varieties of

Kale, however in G M Dari the intensity of certain bands was low as compared to those in Khanyari.

- Band No.2 corresponding to 175kD was present only in T_8 in both the varieties but with low intensity. Band No.3 was present in T_1, T_4, T_3 and T_5 in both the varieties, however with different intensities.
- Band No. 4 corresponding to 83 kD was present in T_8 in both the varieties but with less intensity. Band No. 5 corresponding to 66 kD was common in T_8, T_4,T_3, T_6 and T_7 in Khanyari but in G M Dari this band was missing in T_6 and T_7. However in both these varieties, this band was present with higher intensity in T_4.
- Band No.6 was present in T_4, T_5 and T_7 in both the varieties, however with different intensities. Band No.7 corresponding to 55kD was present in all the treatments in both the varieties but with higher intensity in T_4.
- Band No.8 was present in T_3, T_4, T_5, T_6 and T_7 with different intensities in both the varieties. Band No. 9 corresponding to 37kD was present in all the treatments of both the varieties and with a higher intensity.
- Band No.10 was present in T_4, T_5, T_7 and T_8 in Khanyari with different intensities however, this band was absent in T_7in G M Dari. Band No.11 was present only in T_3 and T_4 in both the varieties.
- Band No. 12 corresponding to 22kD was present in all the treatments in both the varieties but with different intensities. Band No. 13 was present in all the treatments with different intensities in both the varieties, however its higher intensity was seen in T_4.
- Band No. 14 and 15 were present only in T_3 and T_4 but with a higher intensity in T_4 in both the varieties.

6.5 Effect of nano-urea on quality parameters

- Maximum vitamin C content (150.20 g/100 g in Khanyari and 148.30 g/100g in G M Dari) was observed for T_8 treatment while the lowest results (67.50 g/100g in Khanyari and 64.50 g/100 g in G M Dari) were observed for control.
- Same trend was followed by the antioxidant activity which decreased with higher doses of nano-urea. The maximum antioxidant capacity (56.30 mg GAE g^{-1} in Khanyari and 45.60 mg GAE g^{-1} in G M Dari) was observed for T_8 treatment while as the lowest value (31.30mg GAE g^{-1} in Khanyari and 22.60 mg GAE g^{-1} in G M Dari) was observed in control.
- The crude protein content was found to be maximum (18.61 g/100g in Khanyari and 13.24 g/100g in G M Dari) for T_4 treatment whereas the lowest value (6.61 g/100g in Khanyari and 6.42 g/100g in G M Dari) was observed in control.

6.6 Effect of nano-urea on leaf color

- All color values; Lightness L*, a*, b*, Hue angle (°) and chroma(C*) of both the varieties of Kale were significantly different for different treatments. The L* value was higher in control (45.27 in Khanyari and 46.21in G M Dari) for both the varieties, compared to T_4 treatment where the L* value was lowest (39.07 for Khanyari and 39.14 for G M Dari). Negative values of a* represent green and the plants receiving T_4 treatment showed higher value which means greener hue compared to all other treatments. Maximum value (-4.71 for Khanyari and -5.95 for G M Dari) for a* was observed for T_4 treatment in both the varieties while as the minimum value (-7.13 for Khanyari and -8.04 for G M Dari) was seen for control. Positive values of b* represent a higher proportion of yellow color over blue. Lower value (10.77 for Khanyari and 12.26 for G M Dari) was observed for T_4 treatment where a higher concentration of nano-urea was used in the spray solution while the maximum value (15.41 in Khanyari and 16.52 in G M Dari) was observed for control in both the varieties.

- Higher hue angle was observed for T_4 treatment for both the varieties (121.68° for Khanyari and 121.66° for G M Dari) where the value for chroma was (12.76 for Khanyari and 11.32 for G M Dari) respectively while as the lower hue angle (115.74° for Khanyari and 115.70° for G M Dari) was seen in T_2 treatment with the value of chroma (18.03 in Khanyari and 16.57 in G M Dari) respectively for both the varieties.

6.7 Effect of nano-urea on yield parameters

- The yield per plant and yield per hectare were seen to be maximum for T_8 treatment where the concentration of nano-urea used in the spray solution was 125 ppm while as the lowest values were seen in control. Maximum yield per plant (104.10 g/plant in Khanyari and 85.10 g/plant in G M Dari) was obtained for T_8 whereas the lowest value (25.30 g/plant in Khanyari and 17.20 g/plant in G M Dari) was observed for control.
- Likewise, yield per hectare was found to be maximum in T_8 (34.50 t ha^{-1} in Khanyari and 27.10 t ha^{-1}) while as the yield per hectare was found to be minimum (11.80 in Khanyari and 8.20 in G M Dari) in control.

CONCLUSIONS

The results showed that the foliar spray of nano-urea proved to be superior over the commercial urea fertilizer. The nano technique proved extremely efficient in improving various morpho-physiological, biochemical and yield parameters of Kale. A foliar spray using as little concentration as 125ppm nano-urea proved more effective than foliar spray with ordinary urea at 2000 ppm. A foliar spray with 125 ppm nano-urea increased growth parameters by surpassing those achieved by the 2000 ppm soluble urea by upto 100% or more. All the plant growth parameters i.e plant height, number of leaves per plant, leaf area per plant, fresh weight of leaves and dry weight of leaves all were found to be maximum with foliar sprayof 125 ppm of nano-urea i.e 3.25 mL L^{-1} to the plant. The physiological parameters like net photosynthetic rate, stomatal conductance, transpiration rate, net assimilation rate and relative growth rate, biochemical parameters like total phenols and total carbohydrates, quality parameters like vitamin C content, antioxidant activity and yield parameters like yield per plant and yield per hectare were found to be maximum for the lowest concentration of nano-urea used in the experiment i.e 125 ppm. Application of higher doses of nano-urea led to decrease in all these parameters and hence proved to be detrimental. The molecular parameter i.e, the protein profiling through SDS-PAGE showed that the maximum number of protein bands were observed with the foliar application of T4 treatment i.e, 2000 ppm of nano-urea. Other physiological parameters like leaf chlorophyll a, leaf chlorophyll b, total leaf chlorophyll content and leaf carotenoid content , biochemical parameters like leaf N, P, K, Ca and nitrate content and quality parameter like crude protein content were all found to attain maximum values with the foliar application of highest concentration of nano-urea i.e, 2000 ppm, which otherwise is a higher concentration that led to the retardation in morphological, quality and yield parameters of Kale. With respect to the leaf color, the darker color of leaves with a green hue was seen where the highest concentration of nano-urea was used and accordingly the leaves in such treatment were having the higher value for hue angle as compared to all other treatments.

Finally it may be recommended from the outcome of the present investigation that the the foliar application of as little concentration as 125 ppm of nano-urea i.e, 3.125 mL L^{-1} is the optimum dose of this liquid nanofertilizer that should he used for enhancing the plant growth , quality and yield of Kale. This study clearly suggests that the soil application of urea fertilizer can be replaced by nano fertilizer through foliar application which will diminish the soil pollution and enhance soil fertility by improving the physical and chemical properties of soil. The use of nanofertilizer will lower the financial burden on government investment for the production of direct fertiliser because one litre of nano-urea liquid will replace 100 kg of urea; additionally, by lowering the cost of production, it will improve the socioeconomic status of the farming community.

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Appendix-1

Mean weekly meteorological data recorded at Faculty of Horticulture, SKUAST-K

Standard Meteorological week	Date and month	Mean Temperature (°C)		Rainfall (mm)
		Max.	Min.	
16	16 April – 22 April	16.71	6.70	4.12
17	23 April – 29 April	25.14	8.42	0.28
18	30 April – 06 May	23.07	9.5	9.22
19	07 May – 13 May	25.28	8.72	0.94
20	14 May – 20 May	23.35	8.65	2.22
21	21 May – 27 May	28.85	9.01	0
22	28 May – 03 June	23.21	11.05	6.14
23	04 June – 10 June	27.64	12.05	2.25
24	11 June – 17 June	28.35	12.14	0.94
25	18 June – 24 June	31.00	13.20	0
26	25 June – 01 July	31.78	14.28	0.42
27	02 July –08 July	32.77	14.84	0
Mean		29.00	14	2.7

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CERTIFICATE

Certified that all the corrections/amendments as suggested by External Examiner **Dr. Sheikh M. Sultan,** Principal Scientist (Botany), ICAR, Regional Station, Rangreth, Srinagar during Viva-Voce examination held on **05-04-2023** have been incorporated in the manuscript entitled **"Impact of Nano-urea on Morpho-physiological, Biochemical and Yield Parameters of Kale (*Brassica oleracea* var. *acephala*)"** submitted by **Ms. Muzain Mushtaq (Regd. No. MSBS-2020-18)**

(Dr. Sajad Ahmad Bhat)
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