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**Macronutrient application affects morphological, physiological, and
seed yield attributes of *Calendula officinalis* L.**

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ABSTRACT

Ahmad, I., N. Jabeen, K. Ziaf, J.M. Dole, M.A.S. Khan and M.A. Bakhtavar.

Macronutrient application affects morphological, physiological, and seed yield attributes of *Calendula officinalis* L. CJPS XX: xx-xx. Effects of N, P and K

applications alone or in various combinations and ratios were studied on the morphological, physiological, yield attributes and seed production of calendula.

Treatment combinations were control (no NPK application), 150 kg ha⁻¹ N, 80 kg ha⁻¹ P, 150 kg ha⁻¹ K, N + P, N + K, P + K and N + P + K, while in a second experiment,

different ratios of NPK were compared to determine the best combination and ratio of tested nutrients for optimal growth, quality, yield and seed production. Plants supplied

with NPK had vigorous growth, higher leaf total chlorophyll content and flowered earlier with greater flower fresh and dry weight along with improved photosynthetic

performance. Plant biomass and seed yield along with leaf N and K was also higher in plants fertilized with NPK. In the second experiment, application of 200:100:100 kg ha⁻¹

NPK resulted in maximum growth, flowering and seed yield along with higher photosynthetic activity. Leaf area and improved leaf nutrients status was observed at

150:150:150 kg ha⁻¹, while 200:200:200 kg ha⁻¹ NPK increased stomatal conductance, photosynthetic rate, leaf P and flower weights. Results demonstrated that higher level of

nitrogen along with lower level of P and K are vital for quality calendula flower and seed production.

Keywords: Flower quality, NPK application, plant nutrition, seed production

Calendula (*Calendula officinalis* L.), (pot marigold), a member of family Asteraceae (Compositae), is an important aromatic ornamental plant indigenous to Europe (Khan

2011) and is cultivated worldwide as outdoor seasonal annual. In addition to being used as a bedding plant or specialty cut flower in landscape horticulture, calendula is also used in cosmetics and pharmaceuticals (Ercetin et al. 2012; Warner and Erwin 2005).

Production of ornamental plants has become increasingly important and lack of proper fertilization recommendations has emerged as a major constraint in their successful cultivation (Mehran et al. 2008; Starkey and Pedersen 1997). The main objective of using the fertilizers is to replace nutrients that have been depleted in the soil due to leaching or continuous uptake by plants (Younis et al. 2011). Nitrogen (N), potassium (K), and phosphorus (P) are the major components in mineral fertilizers and have a major influence on plant growth when applied in the optimum combinations (Younis et al. 2013).

N, P and K play a pivotal role in production of high quality flowers and seeds of ornamental plants (Kashif 2001). Nitrogen has a greater influence on plant growth, quality and yield than any other element and its application enhances vegetative growth (Kashif et al. 2014). Early maturity of crops and development of roots has been related to availability of phosphorus (Ahirwar et al. 2012). Potassium is associated with opening and closing of stomata, which influence CO₂ uptake for production of photosynthates during photosynthesis. Too much fertilization results in salt accumulation in root zone, which is detrimental for plant growth (Owen et al. 2008). In semi-arid regions like Faisalabad, Pakistan, higher salt accumulation is quite common in soils due to limited rainfall and high evapotranspirational losses (Azeem and Ahmad 2011). Moreover, due to high pH, nutrient availability is limited which demands higher amounts of macronutrient application for best quality flower production.

Not surprisingly, a positive effect of nutrient application (NPK at 80:30:30 kg ha⁻¹) on growth and flower yield of *Calendula officinalis* L. was found (Singh and Godara 2015). Nejad and Shakib (2013) reported that morphological characteristics and flower longevity of calendula was maximum when nitrogen was applied at 150 kg ha⁻¹. Application of nitrogen (urea) and farmyard manure on *Celosia cristata* at 50 kg ha⁻¹ and 300 kg ha⁻¹, respectively, produced the best vegetative growth, flower yield and quality, and seed yield (Yagi et al. 2014). Moreover, morphological and floral attributes of zinnia increased significantly with application of 20 g m⁻² P₂O₅ (Shah et al. 2014).

Enhancement of vegetative growth and flower quality of ornamentals has remained the key focus of researchers. Best quality plant and flower production in areas with high pH soils and semi-arid climates has always been a challenge. Therefore, a study was conducted to investigate the effect of N, P and K alone or in various combinations on vegetative growth, physiological attributes, flowering and seed yield of calendula and to optimize the level of NPK fertilization for calendula flower and seed production.

MATERIALS AND METHODS

Plant Material and Experimental Details

The study was conducted at Rose Project Research Area (31.4336° N, 73.0683° E), Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan, (latitude 31°30N, longitude 73°10E and altitude 213 m) during 2014-15. Nursery stock was raised in 128-cell plastic plug trays containing silt, coco coir and well-decomposed leaf manure (1:1:1; v/v/v) substrate on 23 October 2014, using seeds of 'Lemon' calendula. After site selection, soil was thoroughly prepared, leveled and specified

fertilizer doses were applied in each experimental unit as per treatments. Urea (46% N), single super phosphate (18% P_2O_5), and sulphate of potash (50% K_2O) were used as source of N, P, and K. Calendula seedlings were transplanted in the plots (1.5 m $\times$ 1.2 m) on 22 November 2014 at the 2-4 true leaf stage in 30 cm spaced rows with 30 cm distance between plants.

Experimental Design and Treatments

Soil samples were randomly taken from all experimental units to evaluate physio-chemical properties (pH, EC, organic matter and N, P and K content) of the soil before addition of fertilizer. The study area climate was semi-arid, while soil type was a sandy loam, which had an organic matter content of 0.66%, pH (8.78), EC (2.50 dS m^{-1}), total nitrogen (480 mg kg^{-1}), available phosphorus (9.6 mg kg^{-1}) and exchangeable potassium (200 mg kg^{-1}). Nitrogen was determined following digestion method of Chapman and Parker (1961), using kjeldahl digestion flask to determine total nitrogen. For phosphorus and potassium, samples were digested using method of Yoshida et al. (1976) followed by spectrophotometric determination as described by Chapman and Parker (1961). The study was comprised of two experiments and twenty plants were planted in each experimental unit. Experiment 1 was repeated for two years, while experiment II was conducted once during 2nd year of study. All treatments were replicated three times in a randomized complete block design. In Expt. 1, treatments were control (no fertilizer application), N at 150 kg ha^{-1} , P at 80 kg ha^{-1} , K at 150 kg ha^{-1} , N + P, N + K, P + K and N + P + K. While, in second experiment, different ratios of NPK: 100:100:100 kg NPK ha^{-1} , 150:100:100 kg NPK ha^{-1} , 200:100:100 kg NPK ha^{-1} , 100:150:100 kg NPK ha^{-1} , 100:200:100 kg NPK ha^{-1} , 100:100:150 kg NPK ha^{-1} , 100:100:200 kg NPK ha^{-1} , 150:150:150 kg NPK ha^{-1} , and 200:200:200 kg NPK ha^{-1} were used. Half of the

nitrogen and all of the phosphorus and potassium were applied at the time of soil preparation before transplanting, while the other half of nitrogen was applied 30 days after transplanting. All other cultural practices such as IPM, hoeing, weeding etc. were similar for all treatments during the entire study period.

Measurements

Growth Attributes

Plant height was measured in centimeters with a measuring rod from base to top of the plant. Five plants were randomly selected from each replicate and measurements for length and width were made on the fully expand leaf selected from the middle of the plant. The values of length and width were multiplied by leaf shape coefficient to calculate leaf area in cm^2 according to Carleton and Foote (1965).

$$\text{Leaf area} = \text{Length} \times \text{maximum width} \times 0.68$$

Where, 0.68 = leaf shape coefficient.

Flowering attributes

Production time (days) was recorded by counting the days from date of transplanting to first flower opening. Five plants were tagged from each plot to count number of flowers per plant. Flowers were counted every five days starting after opening of first flower until end of blooming period. Similarly, diameter (mm) of five selected flowers was measured from the tagged plants using digital caliper and average was computed. At full bloom, five randomly selected flowers were harvested and fresh weight (g) of individual flowers was recorded on electric balance. For flower dry weight (g), flowers were packed in paper bags and kept in an oven for 72 hours at a constant temperature of 60°C . After drying to a constant weight, flowers were taken out and dry weight of individual flower was recorded.

Physiological attributes

Two healthy and recently mature leaves were selected from each plant and leaf total chlorophyll content (SPAD) were determined from three points of leaf (leaf tip, middle and leaf base) with digital leaf chlorophyll meter (CCM-200 Plus, Opti-Sciences, Inc., Hudson, NH, USA).

Gas exchange measurements, stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$), transpiration rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$) and photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$) were recorded at mature leaf stage after 60 days of sowing. For this purpose an infrared gas analyzer (IRGA, Lci-SD-ADC Bio-Scientific, UK) was used. Three plants were tagged from each experimental unit and measurements were taken from central part of the leaf blade that did not include midrib. These measurements were recorded from 10:00 - 15:00 on bright sunny days.

Leaf N, P or K analysis

Leaf samples were collected at the start of flowering to determine total leaf nitrogen (Lierop 1976), phosphorus and potassium content. The leaves were oven-dried at 65 °C and grounded to a powder. A sample (0.1 g) of leaf tissue was digested in 2 ml sulfuric acid-hydrogen peroxide mixture as described by Wolf (1982). The amounts of K^+ in the digested samples were determined with a Sherwood-360 flame-photometer. Leaf phosphorus content were measured in digested sample using an UV-Vis spectrophotometer (Lambda EZ 150, Perkinelmer Wallac) by taking absorbance at 420 nm and comparing it with standard curve (Chapman and Parker 1961).

Seed yield attributes

At maturity five selected plants were harvested and dried under the sun for 10 days, and plant dry weight (g), including stem, leaves and flowers were recorded to

estimate the biological yield. Data of seed yield per plant were recorded by manually separating seeds from the flowers and weighing whole seed produced by a plant. Harvest index (%) was calculated by using formula devised by Hunt (1978):

$$HI = \frac{\text{Seed yield(g)}}{\text{Biological yield(g)}} \times 100$$

Weather Data

The research area was located at The University of Agriculture, Faisalabad, Pakistan. Average monthly temperature and relative humidity were recorded during the entire period of study. Maximum temperature (25 °C) was recorded during October 2014 after which temperatures decreased from November to January and then increased up to 19°C in March 2015. RH increased from 55% in October 2014 to 75% in both January and February and then decreased to approximately 64% in March 2015. Average monthly sun shine hours during whole study period were 6.13 hours with maximum 8 hours of sun shine in month of October and minimum 4.7 hours in December 2014. Total rainfall during the study period was 143.6 mm, being maximum during March 2015 (67.9 mm) and minimum during December 2014 (0.0 mm). Six irrigations with good quality canal water were made through surface irrigation to the field capacity as per crop requirement and weather conditions during the study period.

Statistical Analysis

Data were analyzed statistically using Fisher's analysis of variance technique by Statistix 8.1 statistical software. Treatment means were compared using Least Significant Difference (LSD) test ($P \leq 0.05$) (Steel et al. 1997).

RESULTS

Expt. 1: Optimizing N, P, and K (kg ha⁻¹) Application alone or in Various Combinations

Growth attributes

Maximum plant height (63.7 cm) was recorded in plants fertilized with combined application of N + P + K, while shortest plants (28.2 cm) occurred when grown without NPK (Table 1). Plants supplied with N+P had greatest leaf area (23.5 cm²), followed by nitrogen alone (23.3 cm²), while the least leaf area (18.3 cm²) was recorded in plants supplied with phosphorus (Table 1).

Reproductive attributes

After transplanting, early flowering (62 days) was observed in plants supplied with N + P + K, whereas flower initiation was delayed (up to 73 days) in plants grown without fertilizer (control), followed by potassium application (72 days) (Table 1). Highest number of flowers per plant (62) was recorded in plants provided with N+P or N+P+K (60). Least number of flowers (25 each) was produced on plants grown with potassium alone and on controls (Table 1). Highest flower diameter (42.7 mm) was recorded in plants supplied with N + P whereas, plants grown without fertilizer application produced flowers with the least diameter (34.2 mm) (Table 1). Flower fresh and dry weight (1.12 and 0.12 g, respectively) were highest in plants fertilized with N + P + K, while lowest flower fresh weight (0.85 g) was observed in P + K application (Table 1) and flower dry weight was minimum (0.097 g each) in plants supplied with K alone and in plants those grown without fertilizer application.

Physiological attributes

Plants grown with N + P + K had the highest leaf total chlorophyll content (19.1), whereas the lowest (12.9) chlorophyll content were recorded in plants grown

without any fertilizer application (Table 2). Maximum value of stomatal conductance was observed in plants supplied with N + P + K ($5.5 \text{ mmol m}^{-2} \text{ s}^{-1}$), while minimum stomatal conductance was recorded in plants provided with P + K ($2.7 \text{ mmol m}^{-2} \text{ s}^{-1}$) or by phosphorus alone ($2.8 \text{ mmol m}^{-2} \text{ s}^{-1}$) (Table 2). Greatest photosynthetic rate ($6.2 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$) occurred where N + P + K was applied, whereas minimum rate ($3.8 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$) was found in plants fertilized with P + K, followed by phosphorus alone and no NPK application ($3.9 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ each); however, all were statistically similar. Transpiration rate was highest in plants fertilized with N + P and in control ($7.1 \text{ mmol m}^{-2} \text{ s}^{-1}$ each), followed by N + K application ($6.9 \text{ mmol m}^{-2} \text{ s}^{-1}$); however, all three were statistically similar. Lowest transpiration rate ($5.8 \text{ mmol m}^{-2} \text{ s}^{-1}$ each) was observed in plants supplied with P + K and N + P + K (Table 2).

Leaf NPK content

Leaf analysis for nitrogen estimation revealed higher N% in plants fertilized with N + P + K application (2.82%), whereas unfertilized plants (control) had minimum (1.63%) leaf N content (Fig. 1). Higher phosphorus was recorded in plants supplied with N + P (0.37%), while plants fertilized with K had minimum (0.26%) phosphorus content. Highest K% (3.10) was recorded in plants supplied with N + P + K (3.10%) followed by K alone (2.91%), while minimum leaf potassium content (2.44%) were observed in control.

Seed yield components

Highest dry weight was produced in plants fertilized with N + P + K (58.7 g) followed by N + P (54.1 g), while minimum plant dry weight was produced in plants supplied with K and control (46.7 and 46.1 g, respectively) (Fig. 2). N+P+K application

resulted in highest seed yield per plant (15.5 g), while lowest seed yield was observed in control and K applied alone (both 9.9 g) (Fig. 2). Maximum harvest index was found in plants supplied with N + P + K (26.4%), while plants supplied with N + P had minimum harvest index (19.7%), which was statistically similar to P + K (20.4%) (Fig. 2).

Expt. 2. Optimizing Levels of NPK (kg ha⁻¹)

Growth attributes

Fertilizer application at 200:100:100 kg ha⁻¹ produced tallest plants (41.3 cm) followed by 150:150:150 kg ha⁻¹ (40.1 cm) and 100:100:200 kg ha⁻¹ (39.9 cm). Plants fertilized with 100:100:100 kg ha⁻¹ were shortest (29.2 cm) among all treatments (Table 3). Maximum leaf area was recorded in plants fertilized with 15:15:15 g m⁻² (35.3 cm²) followed by 200:100:100 and 200:20:200 kg ha⁻¹ with 34.5 cm² and 32.8 cm² leaf area, respectively. The lowest leaf area (24.7 cm²) was produced by plants supplied with the 100:100:150 kg ha⁻¹ dose of fertilizer (Table 3).

Reproductive attributes

Fertilizer application at 200:100:100 kg ha⁻¹ resulted in earliest flowering (61.3 d) of all treatments, followed by 150:150:150 kg ha⁻¹ (63.8 d). Whereas, plants fertilized with 100:150:100 kg ha⁻¹ took longest time to flower, about 77 days after transplanting (Table 3). Maximum numbers of flowers per plant were due to application of fertilizer at 200:100:100 kg ha⁻¹ (50.5 flowers) followed by 150:150:150 kg ha⁻¹ (49.8 flowers); both were statistically similar. The least numbers of flowers (34.3) were recorded in plants supplied with 150:100:100 kg ha⁻¹ (Table 3). Greatest flower diameter was found in response to 200:100:100 kg ha⁻¹ dose of fertilizer (47.1 mm), while the smallest flower diameter (36.8 mm) was recorded in plants fertilized with 100:100:100 kg ha⁻¹ (Table 3). Highest fresh weight of flowers (0.99 g) was recorded with 200:200:200 kg

ha⁻¹ application followed by 200:100:100 kg ha⁻¹ (0.98 g), while minimum (0.86 g) flower fresh weight was recorded in 150:100:100 kg ha⁻¹ (Table 3). Plants supplied with 200:200:200 kg ha⁻¹ had maximum flower dry weight (0.114 g), while lowest dry weight (0.085 g) was recorded in plants supplied with 100:100:100 kg ha⁻¹.

Physiological attributes

Plants fertilized with the highest N levels (200:100:100 or 200:200:200 kg ha⁻¹) had the highest leaf total chlorophyll content (17.8 or 16.6, respectively), whereas the lowest leaf total chlorophyll content (11.4) were observed in plants fertilized with 100:100:100 kg ha⁻¹ (Table 4). Maximum stomatal conductance was observed in plants supplied with 200:200:200 kg ha⁻¹ (4.35 mmol m⁻² s⁻¹) or 150:100:100 kg ha⁻¹ (4.23 mmol m⁻² s⁻¹). Minimum stomatal conductance (2.32 mmol m⁻² s⁻¹) was recorded in plants fertilized with 100:100:150 kg ha⁻¹ (Table 4). Photosynthetic rate was highest when plants were grown with either 200:200:200 or 200:100:100 kg ha⁻¹ (9.9 or 9.7 µmol m⁻² s⁻¹, respectively). Plants fertilized with 100:100:100 kg ha⁻¹ showed a minimum rate (5.5 µmol m⁻² s⁻¹) (Table 4). Highest transpiration rate (8.5 mmol m⁻² s⁻¹) was recorded in plants fertilized with 100:150:100 kg ha⁻¹, while lowest transpiration rate (5.9 mmol m⁻² s⁻¹) was observed in plants supplied with 200:200:200 kg ha⁻¹ (Table 4).

Leaf NPK content

The highest %N occurred in plants fertilized with 150:150:150 kg ha⁻¹ (2.67%) followed by 200:200:200 kg ha⁻¹ (2.48%) and both were statistically similar, whereas minimum leaf N content (1.67%) was exhibited by the 100:100:100 kg ha⁻¹ application (Fig. 3). Higher phosphorus content was recorded in 200:200:200 kg ha⁻¹ treatment (0.64%) followed by 100:200:100 kg ha⁻¹ (0.60%). Plants fertilized with 150:100:100 kg

ha⁻¹ had minimum phosphorus content (0.28%), which was statistically similar (0.29%) to 100:100:100 kg ha⁻¹ (Fig. 3). Maximum K was recorded in plants supplied with 150:150:150 kg ha⁻¹ (3.06%) followed by 100:100:200 kg ha⁻¹ (2.97%), while minimum leaf potassium content (2.52%) was found with 100:100:100 kg ha⁻¹ (Fig. 3).

Seed yield components

Maximum plant dry weight was recorded with application of fertilizer at 200:200:200 kg ha⁻¹ (62 g) followed by 200:100:100 kg ha⁻¹ (59 g); both were statistically similar. Minimum plant dry weight (51 g) was observed in plants supplied with 100:100:100 kg ha⁻¹ (Fig. 4). Highest seed yield per plant was recorded in response to 200:100:100 kg ha⁻¹ (14.1 g), which was statistically similar to 150:150:150 kg ha⁻¹ (13.7 g), while lowest seed yield (9.6 g) was observed due to application of 100:100:100 kg ha⁻¹ (Fig. 4). Maximum harvest index was exhibited by the plants supplied with 200:100:100 kg ha⁻¹ (23.9%) followed by 150:150:150 kg ha⁻¹ (23.5%), while plants grown with 100:100:100 kg ha⁻¹ had minimum (18.9%) harvest index (Fig. 4).

DISCUSSION

Application of NPK fertilizer combined with higher nitrogen rates significantly improved the growth, flowering traits, physiological attributes, seed yield and leaf nutrients status of *Calendula officinalis*. These results document the importance of nitrogen in vegetative growth, which improved photosynthetic and other metabolic processes and improved flower/yield and seed quality. Nitrogen plays a central role in plant metabolism as a constituent of proteins, nucleic acids, chlorophyll, co-enzymes, phytohormones and secondary metabolites, while phosphorus is involved in energy storage and transfer (Hawkesford et al. 2012). Increased plant height is the most

obvious sign of improved growth, but there was also a significant increase in leaf area of plants supplied with N + P in Expt. 1 and 150:150:150 kg ha⁻¹ in Expt. 2. Nitrogen tends to increase leaf cell numbers and cell size resulting in an overall increase in leaf size. Also, the phosphorus and potassium content in the soil increased by fertilizer application might have also resulted in more chlorophyll formation and more photosynthesis and increased leaf area. Khosa et al. (2011) and Paradhan et al. (2004) demonstrated that application of N + P produced maximum leaf area in *Gerbera jamesonii* L. Moreover, chlorophyll content increased due to fertilizer application, particularly the triple nutrient application of NPK in first experiment and the 200:100:100 kg ha⁻¹ treatment in the second experiment. These results are in line with the findings of Skwarylo-Bednarz and Krzepilko (2013). This increase in photosynthetic capacity is likely due to the fact that proteins of the Calvin cycle and thylakoids represent the majority of leaf nitrogen (Gibson 2005). Similarly, N, P and K application also resulted in maximum leaf total chlorophyll content in *Tulipa gesneriana* (Ali et al. 2014).

Triple nutrient treatment (N+P+K) proved the most effective, especially when associated with higher N and P rates, for increasing flower fresh and dry weight and flower diameter along with reducing the crop duration by early flowering. As a result the plants produced more photosynthates, which resulted in higher flower yield and early flower production (Chadaha et al. 1999). The most suitable combination of NPK to achieve minimum production time was 200:100:100 kg ha⁻¹, which also resulted in maximum number of flowers per plant and greatest flower diameter. Previously, Alkurdi et al. (2014) also observed that nitrogen and phosphorus were helpful in increasing number of flowers and facilitated flower bud opening.

Gaseous exchange is directly linked to physiological processes like photosynthesis and transpiration. Application of N, P and K greatly increased stomatal conductance and photosynthetic rate, while N + P application gave higher transpiration rate in Expt. 1. Similarly, in the second study, 200:200:200 kg ha⁻¹ was identified as most suitable fertilizer combination for maximum stomatal conductance and higher photosynthetic rate, while transpiration rate was higher with 100:150:100 kg ha⁻¹. These results suggested the importance of potassium in the fertilizer as stomatal movements are reported to be regulated by potassium (Taiz and Zeiger 2006).

Leaf nutrient analysis showed higher N and K content in plants supplied with NPK, while plants fertilized with N+P had higher P content in Expt. 1, due to increased absorption, translocation, and accumulation of applied nutrients in leaf tissues. In the second study, 150:150:150 kg ha⁻¹ was identified as most suitable treatment to improve leaf N and K content, whereas tissue phosphorus was higher from the 200:200:200 kg ha⁻¹ treatment. Being a shallow rooted crop, plants required more nutrients available around root zone. Moreover, plant roots may be unable to absorb nutrients present in the soil due to higher pH of the soil and higher amounts of macronutrients performed better due to more availability. Similar results have been reported by Sarwar et al. (2013) in *Matthiola incana* in which application of 1.5% NPK increased the leaf nitrogen (6%), potassium (38.1 ppm) and phosphorus content (0.46 ppm). Zhang et al. (2012) observed increased accumulation of dry matter and leaf NPK content in *Petunia hybrida* with the combined application of NPK.

Addition of adequate amount of fertilizer in the soil increased plant biomass. Higher doses of NPK enhanced number of leaves and branches, which ultimately

increased plant dry weight (Kashif et al. 2014). Similarly, N, P and K fertilization increased dry weight of plant in *Dahlia hybrida* (Alkurdi 2014).

Flower fresh and dry weights are important indicators of potential seed production. Combined application of NPK was best for flower fresh and dry weight and most suitable combination was 200:200:200 kg ha⁻¹. Application of all required nutrients in appropriate quantities to the plant might have enhanced photosynthate production, allowing the production of more and larger flowers with higher weights (Ahmad et al. 2011). Similar findings have also been reported in *Zinnia elegans* (Javid et al. 2005). Maximum seed yield was also observed with the combined application of NPK, which was statistically superior to all other treatments in first study. While in second study 200:100:100 kg ha⁻¹ was identified as best combination of NPK for maximum seed yield. This indicates that there is a positive link between seed yield of calendula and application of a complete NPK fertilizer as nitrogen is a primary constituent of proteins (Rahmani et al. 2009) and phosphorus promotes reproductive growth through its significant role in energy transfer and storage for plant metabolism. Increased harvest index is due to increased seed yield of the plants that were supplied with combined application of NPK as there is a positive correlation between seed yield and harvest index (Synder and Carlson 1984).

CONCLUSION

In conclusion, inorganic fertilizers containing NPK combinations and amounts are obligatory for optimum growth, flower quality and seed production of calendula. Plants fertilized with NPK or N alone performed the best in terms of plant growth, while reproductive attributes were most positively influenced by the application of N + P at 150 + 80 kg ha⁻¹. In Expt. 2, NPK at 200:100:100 kg ha⁻¹ performed the best for

increasing growth, early flowering and high seed yield. Results demonstrated that combined NPK application with a higher rate of nitrogen is vital for quality calendula flower and seed production and growers may use 200:100:100 kg ha⁻¹ NPK to produce optimal flower and seed yield of *Calendula officinalis* L., an economically important bedding and aromatic plant. Therefore, calendula growers should not just focus on nitrogen application; rather they should also apply P & K to increase flower and seed quality. Moreover, it appears that the high pH reduces native soil nutrient availability, necessitating application of considerable amounts of nutrients. Further, the impact of applied nutrient doses on seed quality as well as oil quantity and quality need to be evaluated to accomplish more benefits of the present study.

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For Review Only

Table 1. Effect of NPK application alone or in various combinations on plant height, leaf area, production time, number of flowers per plant, flower diameter and flower fresh weight of *Calendula officinalis* L.

Treatments (kg ha ⁻¹)	Plant height (cm)	Leaf area (cm ²)	Production time (days)	Number of flowers per plant	Flower diameter (mm)	Flower fresh weight (g)	Flower dry weight (g)
Control (0)	28.2 d ^a	19.1 bc	73 a	25 c	34.2 d	0.91 bc	0.097 c ^a
Nitrogen (150)	55.7 b	23.3 a	63 bcd	51 ab	39.9 abc	1.09 a	0.106 abc
Phosphorus (80)	29.7 d	18.3 c	69 ab	33 c	37.0 bcd	1.02 ab	0.102 c
Potassium (150)	28.6 d	19.3 bc	72 a	25 c	36.4 cd	0.92 bc	0.097 c
N+P (150+80)	44.0 c	23.5 a	63 bcd	62 a	42. 8 a	0.92 bc	0.114 ab
N+K (150+150)	54.5 b	20.7 abc	63bcd	48 b	41.0 ab	1.09 a	0.104 bc
P+K (80+150)	29.6 d	19.8 bc	68 abc	28 c	39.7 abc	0.85 c	0.108 abc
N+P+K (150+80+150)	63.7 a	21 7 ab	62 d	60 a	40.9 ab	1.12 a	0.116 a
Significance ^b	< 0.0001	0.0077	0.0027	< 0.0001	0.0082	0.0090	0.0139

^aMean separation within columns by Fisher's LSD at $P \leq 0.05$. Values represent means of 15 plants.

^b P values were obtained using Statistix 8.1 (analytical software) for significant effect of fertilizers combinations.

Table 2. Effect of NPK application alone or in various combinations on flower dry weight, leaf total chlorophyll content, stomatal conductance, photosynthetic rate and transpiration rate of *Calendula officinalis* L.

Treatments (kg ha ⁻¹)	Chlorophyll content (SPAD)	Stomatal conductance (mmol m ⁻² s ⁻¹)	Photosynthetic rate (μmol m ⁻² s ⁻¹)	Transpiration rate (mmol m ⁻² s ⁻¹)
Control (0)	12.9 c	3.6 bc	3.9 c	7.1 a
Nitrogen (150)	16.5 b	4.6 ab	5.5 ab	6.8 ab
Phosphorus (80)	13.4 c	2.8 c	3.9 c	6.1 bc
Potassium (150)	13.6 c	3.7 bc	4.6 bc	6.2 bc
N+P (150+80)	16.8 b	4.7 ab	4.7 bc	7.1 a
N+K (150+150)	16.4 b	4.1 abc	4.3 bc	6.9 a
P+K (80+150)	13.6 c	2.7 c	3.8 c	5.8 c
N+P+K (150+80+150)	19.1a	5.5 a	6.2 a	5.8 c
Significance ^b	0.0002	0.0350	0.0308	0.0148

^aMean separation within columns by Fisher's LSD at $P \leq 0.05$. Values represent means of 15 plants.

^b P values were obtained using Statistix 8.1 (analytical software) for significant effect of fertilizers combinations.

Table 3. Effect of various levels of NPK application on plant height, leaf area, production time, number of flowers per plant, flower diameter and flower fresh weight of *Calendula officinalis* L.

Treatments	Plant height	Leaf area	Production time	Flowers per plant	Flower diameter	Flower fresh	Flower dry
NPK (kg ha ⁻¹)	(cm)	(cm ²)	(days)	(no.)	(mm)	weight (g)	weight (g)
100:100:100	29.2 b ^a	26.6 de	71.4 ab	37.4 bc	36.8 d	0.88 cd	0.085 d ^a
150:100:100	31.2 b	31.6 bc	70.6 abc	34.3 c	41.5 bc	0.86 d	0.089 cd
200:100:100	41.3 a	34.5 ab	61.3 d	50.5 a	47.1 a	0.98 ab	0.105 ab
100:150:100	30.7 b	28.6 cd	76.6 a	36.0 bc	40.7 bc	0.93 bcd	0.101 bc
100:200:100	35.2 ab	25. 9 de	70.9 abc	37.5 bc	43.3 abc	0.92 bcd	0.101 bc
100:100:150	35.7 ab	24.7 e	70 .0 abc	35.5 bc	43.5 abc	0.88 cd	0.094 bcd
100:100:200	39.9 a	31.3 bc	72.1 ab	36.5 bc	40.1 cd	0.93 bcd	0.093 bcd
150:150:150	40.1 a	35.3 a	63.8 cd	49.8 a	44.7 ab	0.93 abc	0.103 ab
200:200:200	35.9 ab	32.8 ab	66.0 bcd	43.1 ab	44.8 ab	0.99 a	0.114 a
Significance ^b	0.0153	0.0001	0.0131	0.0051	0.0055	0.0105	0.0046

^aMean separation within columns by Fisher's LSD at $P \leq 0.05$. Values represent means of 15 plants.

^b P values were obtained using Statistix 8.1 for significant effect of various NPK levels.

Table 4. Effect of various levels of NPK application on flower dry weight, leaf total chlorophyll content, stomatal conductance, photosynthetic rate and transpiration rate of *Calendula officinalis* L.

Treatments	Chlorophyll content	Stomatal conductance	Photosynthetic rate	Transpiration rate
NPK (kg ha ⁻¹)	(SPAD)	(mmol m ⁻² s ⁻¹)	(μmol m ⁻² s ⁻¹)	(mmol m ⁻² s ⁻¹)
100:100:100	11.4 e	3.22 ab	5.5 d	7.5 ab
150:100:100	14.9 bcd	4.23 a	8.6 ab	6.7 bc
200:100:100	17.8 a	3.39 ab	9.7 a	7.4 ab
100:150:100	12.5 de	3.50 ab	6.3 cd	8.5 a
100:200:100	14.4 bcd	2.68 b	6.2 cd	6.8 bc
100:100:150	15.2 bc	2.32 b	6.5 cd	7.7 ab
100:100:200	13.6 cde	2.42 b	7.8 bc	6.9 bc
150:150:150	15.9 abc	3.40 ab	8.7 ab	6.4 bc
200:200:200	16.6 ab	4.35 a	9.9 a	5.9 c
Significance ^b	0.0015	0.0431	0.0030	0.0453

^aMean separation within columns by Fisher's LSD at $P \leq 0.05$. Values represent means of 15 plants.

^b P values were obtained using Statistix 8.1 for significant effect of various NPK levels.

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Fig. 3. Effect of various levels of NPK application on leaf nitrogen (A), phosphorus (B) and potassium content (C) of *Calendula officinalis* L. Bars represent means of 15 samples $\pm$ S.E.

Fig. 4. Effect of various levels of NPK application on plant dry weight (A), seed yield per plant (B) and harvest index (C) of *Calendula officinalis* L. Bars Represent means of 15 samples $\pm$ S.E.

Fig. 1

Fig. 2

Fig. 3

Fig. 4