Systematic prompting the new physiological and morphological, biological responses of modified metabolic induction process of *Calendula officinalis* L under heat stress with couple of supplementary nutritional applications as safety of floriculture crops

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Abstract

Purpose

the objective of study to modified the metabolism of *Calendula officinalis* L. under heat stress couple with N and P application in two years of study.

Methods

the trail was conducted during peak summer season (months of May, June, and July) couple with different levels of N & P application to monitor the quality and quantity characterization of marigold (*Calendula officinalis* L.). The traits of three doses of N and P (0.6 g, 0.9 g, and 0.8 g, 1^{-1} g) given to *Calendula officinalis* L.) to display the vegetative, reproductive, physiological parameters such as Malondialdehyde (MDA), Chlorophyll contents, Lipid Peroxidation Assay in leaves, Li and K contents, phytochemicals and nitrogen and phosphorus used efficacy

Results

showed that maximum values of Malondialdehyde (MDA), Chlorophylls, Lipid Peroxidation Assay in leaves observed followed by the alteration in Li and K measured in the month of May, June with slight differences July. The phytochemicals like total phenolic contents (84.41 mg GAE/g), total antioxidants (36.3% DPPH), total carotenoids and total flavonoids contents (16.2 and 0.9 mg/100 g) were measured by 0.9 g of Nitrogen application and followed by 1 g of Phosphorus in both years of study. The respirational changes were observed in the higher rate of P levels. The liner changes of N and P rates showed some fluctuation in heat stress months.

Conclusions

It was concluded that the higher doses of both N and P fertilizers were effective in controlling the heat stress and mentioned the quality of florets by various induction and biological active process.

Introduction

Horticultural crops play a vital role in international trade and getting incentive in our country for as source of earing (Khan, 2001). The floriculture crops had impacts on the development of business in all over the world through their highly demands values (Khan, 2001). Medicinal and ornamental plant like *Calendula officinalis* L, contained significant bioactive compounds (Pérez Gutiérrez *et al.*, 2006). belongs to family Asteraceae (Khan, 2001) although its origin is west Asia and Mediterranean of the world (Mozafariyan, 2003) but also cultivated in 17th century as an herbal medicine in Europe followed by its cultivation in growing areas of Germany, Czech Republic, Slovakia, Austria and Switzer Land, Hungary and recently in Egypt and Syria due to its significance (Paul *et al.*, 2002). *Calendula officinalis* L, is the rich resource of essential oil, triterpene saponins, flavonoids, carotenoids, saponins, polyacetylenes, sterols, carbohydrates, vitamin C, mucilages and minerals, e.g., manganese (Grant *et al.*, 1985). The physiological responses of Photosynthetic function is recognized as one of the most sensitive indicators of physiological processes to elevated temperatures changes in marigold flower the critical months are seriously issue in which the stomatal and non-stomatal gaseous exchanges under heat stress months (Paul *et al.*, 2002). The heat stress was alter the stomata closure to reduce water loss, altering stomata and trichome density are the most common anatomical changes observed under high environmental temperatures in floriculture crops, the heat stress is a critical issue the high-

temperature stress during summer months severely reduces the marketable quality and landscape survival capacity of marigold, the reduction in the flower diameter, and flower number in calendula has been reported with an increase in temperature (Warner & Erwin, 2005). The results of our previously reported study also showed that morphological characteristics (plant height, leaf area, shoot and root weight, flower diameter, flower number and flower longevity) of calendula cultivars were reduced by high temperature stress, especially in prolonged exposure. However, the extent to which growth and flowering parameters were reduced differed among cultivars. High ambient temperatures causes heat stress and is a serious problem of the agricultural world (Hall, 2001). Temperature stress even high or low, are significant environmental factors that influence growth, development and physiological and biochemical structure of floriculture plants (IPCC, 2007). It speeds up the kinetic energy and movement of molecules in the membrane. The lipid bilayer produced more fluid either denaturing of protein, results an increased in the contents of fatty acid (Savchenko et al., 2002). Secondary metabolites play a role in tolerance during abiotic stress condition (Wahid, 2007). Stress protein, chemicals, and modification of hormonal are more prominent for supplemental application of nutrients (Mozafariyan, 2003; Liu et al., 2006; Feussner et al., 1997). Chemical fertilizers like Phosphate fertilizers put impact on growth of *Calendula officinalis* L (Mozafariyan, 2003) as quality and quantity of plants is effected by nutrients (Paul et al., 2002).

In stress condition presence of nitrogen plays a very crucial role to overcome the pressure of environment (Huang et al., 2004) in prevention of the photo oxidative damages (Kato et al. 2003; Waraich et al. 2011; Zhang et al., 2006). The nitrogen is considered as a muscularly mobile nutrient in a plant that moves with living cells, and its deficiency caused a problem in flowering and quality of plants (Marschner, 1995; Olson and Swallow, 1984). Phosphorous plays a dynamic part in minimizing heat stress and available to plants as an orthophosphate ions. It is a mobile nutrient in plant and transferred to sites of new growth and promotes the plant growing structures like (Cell division, cell elongation). Reduced crop yield observed under N deficiency, whereas supplements of N fertilizers can considerably upsurge productivity of the plants (Olson and Swallow, 1984; Grant et al., 1985 ; Olson and Swallow, 1984). The application of N fertilizers increased growth as it is primary constituent of proteins and are extremely liable to a loss in the range of 20 to 50% for dry matter production (Olson and Swallow, 1984 ; Grant et al., 1985). The electron flux such as Li and K play a vital role in abiotic stress condition, these fluxes have potential to attained a particular ion exchange capacity these ions should be bound to the heat radiation structure and help for heat tolerance mechanism in flowers and removed the heat in any part of plants (Grant et al., 1985).

The aims and objective of this article to explore active the role of induced modified mechanism of N & P application on quality, quantity and physiological responses under heat stress environment of marigold (*Calendula officinalis* L) with advance monitoring and evaluation process. The non-reports are available on marigold nutritional requirements supplementary dozes with active nutritional responses under abiotic stress condition with trigger responses in metabolism changes under heat stress and its control through N and P fertilizers reported for the first time up to my best knowledge with modern and advances modelling and biocenology methods was performed in this study. The Systematic prompting the new physiological and morphological, biological responses of modified metabolic induction process of *Calendula officinalis* L under heat stress periods couple with supplementary nutritional applications as safety of floriculture crops with efficient modelling of Potential nitrogen mineralization (PNM) of media were reported in the relevant section of this article and discussed with all necessary physiological responses under altered metabolism changes during stress periods with advanced biotechnology approaches and methods.

Materials And Methods

Experimental site and location:

The growing condition of *Calendula officinalis* L. were monitored in current investigation under stress at Horticultural Nursery, The University of Haripur, and Khyber Pakhtunkhwa, Pakistan. It is located 34.00 latitude and 72.93 longitudes and it is situated at elevation 537 meters above sea level.

Seed sowing in pots with modern monitoring system

The diameter of plastic container 200 mm deep was used, and the seed of *Calendula officinalis* L was shifted into the pots with a mixture of different media shown in Table 1. The solarization of media was done for removal of any contamination before shifting into pots.

Media Preparation with active materials

The seeds were planted in a medium possess a cocopeat and sand (40:40 v/v) shown in Table 1. After 47 days of planting the seedling in four leaves stage were transplanted into large flower pots with the mixture of sand mould and cocopeat ratio of (1:1:1).

Treatments and Responses under stress periods

The improvements of florets quality of marigold was planned to monitor under N and P doses in heat stress conditions where different levels of N and P (N₂ 0.6 & 0.8 g and P₂ 0.9 & 1 g) per pot were applied at four leaves stages of plant to evaluate the responses of these foliar sprays. The physiological responses of these fertilizers under the heat stress condition of Pakistan were implemented according to the growing periods of marigold.

Vegetative and Reproductive growth of Marigold flowers under hot climatic condition

Measurements of vegetative characters like plant height determined via a ruler at the end of the experiment by cutting above ground parts at the soil surface. Leaves, branches, petals numbers were also counted at the same time. Flower size and leaves length were calculated by a digital caliper (guanglu model) from complete flowers.

The weight of dry flower and leave was determined by following formula:

$$\text{Dry matter (\%)} = \frac{\text{Dry weight}}{\text{Fresh weight}}$$

Physiological Parameters of marigold under stress changes

Preparation of Malondialdehyde (MDA) and Calibration Standards

The calibration curve for determination of concentration of MDA (1 mM) prepared in glacial acetic acid by dissolving the MDA (31.35 mg) in 100 mL solvent followed by the preparation of different concentrations of 0.1, 0.2, 0.4, 0.6, and 0.8 mM to prepare standard curve.

Malondialdehyde (MDA) contents per gram FW (MDA)

Two dissimilar extract prepared, using 100% pure acetic acid and mixed 50% acetic with 50%water. The 1 mL solution of TBA and 1 mL standard MDA was taken in a 10 mL test and mixed then heat on water bath at 95°C for 60 minutes followed by cooling at room temperature. The absorbance of the mixture recorded at 532 nm UV/Visible spectrophotometer, a blank sample of acetic acid or water was also run. The calibration curve prepared through standard solutions. The concentration of MDA calculated through from the calibration curve.

Chlorophyll contents

Chlorophyll contents were measured in leaves (100 mg) and crushed into motor piston in acetone (80%) followed by centrifugation about 2000 rpm done for 3 mins. until colorless filtrate obtained. Absorbance of the filtrate recorded with blank 80% acetone on (UV-1800A) Shimadzu spectrophotometer. Pigment contents were determined by the method of Ahmed et al., 2018.

Determination of K⁺ contents of membrane

The advanced technique Inductively coupled plasma (ICP) optical [emission spectrometry](#) used to measure the K⁺ content in leaves which were dried in oven at 70 °C for 48 h and then agitated in 5% H₂SO₄ overnight followed by the determination of K⁺ content using standard curves.

Flux membrane stability of Li⁺ and K⁺

The [Net fluxes](#) of Li⁺ and K⁺ were measured in leaf samples using the NMT technique by moving microelectrodes and calculated by Fick's law of [diffusion](#) (Saadalla *et al.* 1990).

Analysis of Phytochemical of marigold flower under heat stress periods

Total phenolic contents (TPC)

Total phenolic contents (TPC) was measured using a Folin-Ciocalteu assay method where 1 mL of Folin-Ciocalteu reagent added into a diluted to 5 times in which 0.2 mL of sample and 0.8 mL 7.5% Na₂CO₃ was added. The mixture were kept in dark for 20 mins then absorption was recorded at 765 nm against control sample (Stintzing *et al.* 2005). The gallic acid used to prepare the standard curve for determination of the concentration in mg equivalent of gallic acid (GAE) per gram.

Determination of DPPH radicals scavenging activity

Brand-Williams et al. (1995) method applied for the determination of DPPH radicals scavenging activity using with the 4 mg DPPH dissolved in 100 mL of methanol and kept in dark place. The different concentrations were mixed with 5 mL of 0.004% DPPH solutions. The absorbance was measured at 517 nm after 20 minutes of reaction against the corresponding blank solution. The assay was performed in triplicates reactions.

Percentage inhibition of free radical DPPH was calculated based on control reading by following equation

$$\text{DPPH scavenged (\%)} = \frac{(A_{\text{con}} - A_{\text{test}})}{A_{\text{con}}} \times 100$$

A_{con} - is the absorbance of the control reaction

A_{test} - is the absorbance in the presence of the sample of the extracts.

Responses of Nitrogen and phosphorus used efficiency methods in heat stress period

The total nitrogen analyzed by the Kjeldahl method (Ladha *et al.*, 2005) using 1 g leaves sample, digested with a mixture of H_2SO_4 – H_2O_2 . The phosphorus content was determined calorimetrically in digested samples with a solution of perchloric acid (72%), ammonium molybdate (5%, aminonaphtholsulphonic acid (0–2 %), 0.5 g of sodium bisulphite and 6 g crystalline sodium sulphite. The total N and P contents determined through method given by (Dobermann, (2007)).

Potential nitrogen mineralization (PNM) of media monitored and evaluation process

The sample media was incubated under waterlogged at 30°C in a controlled temperature cabinet for 11 days (a 2: 1 water: media ratio was maintained during incubation). Three models, i.e. the single first-order kinetics model, the double first order kinetics model and the mixed first-order and zero-order kinetics model were used to simulate the cumulative mineralized N (NH_4 + -N and TSN) in the laboratory and waterlogged incubation. The complete model of PNM shown in Table 5.

Analysis of Growth media an nutritional profiling in growth and developmental stages

The analysis of growth media comprises of total organic matter and total nitrogen of the soil. For total nitrogen of soil sample Kjeldahl method was used where H_2SO_4 used for titration. Organic matter estimated by dissolving 1g air-dried soil sample in 10 ml 1N potassium dichromate solution and 20 ml concentrated sulfuric acid in a 500 ml beaker, using a dispenser to mix the suspension for 30 minutes, then 200 mL of distilled water and 10 ml concentrated orthophosphoric acid added followed to cool it. and mixture was allowed to cool. Titration of the mixture carried out by ammonium sulfate solution using diphenylamine

Responses of Respirational changes under heat stress condition

The respirational rate was determined using a portable infrared gas analyzer 6250, LI-COR, Inc., Lincoln, Nebr.), connected with automatic software to record the reading during critical months.

Statistical Analysis

A complete randomized block design pot experiment conducted with various concentrations of N and P followed by the monitoring of their interaction under stress condition with the plants by analysis of variance (ANOVA). The post-hoc test with student newman Kuehl's (SNK) multiple-range test (* $p < 0.05$) were calculated. All samples were normal, and homogeneous variances were tested using the Levene and Cochran tests were implemented on SPSS 21.0 Inc., Chicago, USA software. The mineralization process was measured from the cumulative amounts of NH_4^+ -N with non-linear regression procedure.

Results And Dissuasion

Morphological and metabolism responses under growth and developmental changes by N & P doses

It was observed that the growth of marigold plant showed modification under N and P application where substantial variations reported in height and number of leaves of plant in comparison with control plant (Table 2) under applied 0.9 g dose of N_2 (8.60 cm and 15.44 respectively) while plant cultivated with phosphorus plant reflect height of 14.22 cm over control. Variations in fresh weight of plants were observed among the fertilizer applications, and the differences were statistically significant as shown in Table1. Fresh weight (72.24g), leaf length (9.86 cm), numbers of flowers (5.88), and the number of branches (8.1) were increased significantly by nitrogen application over control. The plants which were applied with nitrogen (0.9 g), and phosphorus (1 g) showed maximum values (6.15 cm) and (5.09

cm) respectively as compared to control (Fig. 1). Vegetative characters like growth and plant height increases under nitrogen application as reported by Bielski and Beata Szwejkowska (2012). N is biologically supplier of H, C, O and S elements as these are primary contents used in the synthesis of the amino acid and protein contents in growing part of flowering plants (Ladha et al. 2005 ; Cassman et al. (2002 ; Husaain et al. (2011); Szwejkowska and Stanisław Bielski, 2012). N improves the quality and quantity attributed to the flowering plants due to continuously increase the cell assimilates and involve in more cell division consequently increase number of leaves of flowering plants (Abdul Kareem *et al.* (2014). Pandey and Mishra, (2005) discloses that the application of N and P improves the vegetative and reproductive parts of flowers. Literature search showed that marigold flower showed improved growth under N application Hazarika et al. (2000). The current investigation is in accordance with the findings of Beata Kraol (2011) who reported, that the application of 80 kg N×ha⁻¹ of nitrogen showed remarkable intensification in raw material yield of the plant while the yield of flower heads did not vary noticeably for same dose of fertilizers Hussein *et al.* (2011) demonstrated that significant difference in the flower diameter between the treated and controls treatments of marigold with doses of N 1.5 and 3.0 g per pot.

Evaluation and monitoring of physiological changes under the application of N& P at heat stress months

The physiological parameters showed significant differences between treatments during the study of 2015-16 in three different heat stress periods like (May, June, and July) (at $P>0.05$) shown in table 3. In the month of May the maximum contents of Malondialdehyde (MDA) under the application of 1 g of P in comparison of control (120 per g FW). Higher contents of Chrophylls were noted in N application followed by P and lower contents of Chrophylls were measured in control. A similar trend of Lipid Peroxidation Assay per gram FW and Li and K contents Flux membrane stability (mg dm⁻³) were measured by application of 1 g of P. However in this month lower contents were noted in control. Higher values of Malondialdehyde (MDA) 155 per g FW recorded in the month of June by the application of 1 g of P in comparison to control. Similar trends were showed by the use of N₂ 0.9 g of Chrophyll contents in leaves of marigold. Lipid Peroxidation Assay per gram FW was higher in June month by application of 1 g of P. The net higher contents of Malondialdehyde (MDA) under 1 g of P in the month of July was observed in comparison of Malondialdehyde (MDA) contents noted in control. Similarly, higher contents of Chrophylls were measured in both months of June and July. Slight variations in Lipid Peroxidation Assay per gram FW and Li and K content Flux membrane stability (mg dm⁻³) were observed in the month of July. Several herberous and succulents' plants are severely damaged by heat stress. The results indicated in Table 3 maximum contents of Malondialdehyde (MDA), as to the signal responses of root to shoot of marigold (Mehmet *et al.*, 2009). This signal mechanism improves by the application of supplementary nutrient supply to the flowering plants Rahbarian et al. (2010).

Heat stress in months of June and July is a serious problem in floriculture industry throughout the world (Fini et al. (2011). In months of July high Lipid Peroxidation Assay per gram FW and Li and K contents Flux membrane stability (mg dm⁻³) due to the response of heat stress in different parts of marigold were observed. These fluxes are stable in the membrane (Yin *et al.*, 2008). The study revealed that the physiological responses under heat stress condition are related to the quality of marigold by the application of nutrients in stress months. N and P involve in reverting the process in plants through continuous adding in different parts of flowering to save the organs and injured parts of flowering plants (Király and Czövek, 2002). The study showed the linked with respiration and physiological response for production of dry matter indispensable to evaluate the significant increase of N uptake for better flowers quality and yield (Yin *et al.*, 2008).

Marigold plants faced problem of lower flowers production and poor quality under heat stress conditions due to its internal structural changes and cellular components. The reactive oxygen species (ROS) caused the internal membrane damages and losses of membrane stability during heat stress conditions (Jain et al., 2001). The high level

of lipid peroxidation related to the MDA content, against heat response observed in marigold organs, clearly indicated that the oxidative stress generated under the effects of a high heat stress period. Several crops (Maize, wheat cotton) under heat stress showed an increased the contents of MDA (Jain *et al.*, 2001; Király and Czövek, 2002; Sofo *et al.*, 2004; Yin *et al.*, 2008).

Chlorophyll is a green pigment and obligates part in photosynthesis activity of plants and considered as a food supply component of flowering plants (Jain *et al.*, 2001; Gupta *et al.*, 2017). Heat stress period is a critical part for photosynthetic pigments. The structures may damage and reverse food in stored parts of organs are lost. The N and P nutrients play a vital role to minimise these oxidative damages Falk *et al.* (1999). The application of these additional nutrients through fertilizers improved the carbon exchange activities in flowering plants and enhanced the pigments of chlorophyll in all parts of plants (Sofo *et al.*, 2004).

Application of N& P on Phytochemicals changes of Pot marigold responses with advance biological changes

The higher contents of total phenolic compound (84.41 mg GAE/g), total carotenoids (16.2 mg/100g), and Total flavonoids content (0.90 mg/100g) and total antioxidants (36.3 %DPPH) were noted in N₂ 0.9 g application over control ($P>0.05$) shown in table 4. Phytochemical play a vital role in oxidative stress (Jain *et al.* 2001). The higher content of Phenyl ammonia lyase and decreased peroxidase activity by the application of nutrient in oxidative stress periods was similar to report of Rivero *et al.*, (2001). The phytochemicals are produced in roots and translocate into the other parts of flowering plants. The current study showed that the enhance contents of these phytochemicals produce in root after application of these nutrients ions, and transferred into leaves, nodes, internodes, buds and final involves in covering the injured organ couple with removal of heat stress injuries in plants (Jain *et al.* 2001). Application of N and P play a role in oxidative stress and a significant role in heat stress months in the flowering plants which develop the ability to tolerate heat stress condition (Falk *et al.* 1999).

Net changes of Dry weight of flower and leaves responses of marigold

The dry weight of flowers and leaves were presented in Fig 3. The higher number of Flowers noted under 1 g of P application over control. The higher number of dry leaves was observed in 1 g of P application. The fact is evident that the higher rate of phosphate fertilizer showed maximum contents of florets parts and leaf materials (Jain *et al.*, 2001; Gupta *et al.*, 2017). Sofo *et al.*, (2004) also report that the dry weights were increased due to extra supply of nutrients.

Advancne modeling of net rate of N and P Efficiency up take induction process of marigold:

The net efficiency rate of N and P of marigold shown in Fig 4. The net rate of N and P efficiency was increased due to a higher supply of nutrients of both doses of N and P. The higher content of both rates were measured by the application of (N₂ 0.9 g and P₂ 1 g). While in Fig 5 showed that N rate of efficiency was lower before application of N supply as compared to after application to marigold. The higher contents of Net N rate efficiency were recorded in 0.9 g of N₂ followed by 1g of P over control, and minor increased is reported after application. The similar trend in Phosphate fertilizer reported in earlier reported work (Dobermann, 2007). The nutrient supply increased the NUE and PUE after application of N and P (Cassman *et al.*, 2002; Dobermann, 2007). The amount of N increased after estimation 5 to 20 % in different crops (Howarth *et al.*, 2002) and 52% (Janzen *et al.*, 2003). Application of N improves the NUE in cereals crops Dobermann, 2007).

Plant responses of Net rate of NUE and PUE under Heat Stress Periods its supply and changes

The net rate of NUE and PUE under heat stress conditions represented in Fig 5, 6 ,7. It was found that the high doses of both N and P were effective on metabolism of the plant in the Month of May which was (1.4%) under 0.9 g of N₂

followed by the (14.5%) in the month of June. While PUE was 32% under 1 g of P application. The month of July, the most critical period of heat stress where the assembles rate of N efficacy rate was (1.7%), over Phosphate application which showed a maximum rate increased (34%) over control for both N and P net rate efficiency.

The summer season is critical for plants growth due to high temperature. The additional application of these nutrients plays a role in minimizing heat stress in these months as reported by Ladha et al. (2005) Cassman et al. (2002), Ladha et al. (2005), Syers *et al.*, 2008). The results were shown in Fig 6. The liner changes of N net rate efficiency showed a value of $r^2 = 0.9641$ in both months of (May and June) while in July the net N rate efficacy value was 0.592. The Net P rate efficiency showed 0.6413 in the month of May, however in June 0.8232 and July was 0.8333. The liner changes showed that maximum variations were recorded in these months.

The Nitrogenous and Phosphates fertilizers had potential role in heat stress periods (Hasanuzzaman et al. 2013). Syers et al., (2008) reported that the heat stress suddenly damages the root system and affect the tissues of xylem and phloem of flowering plants while application of fertilizer neutralizes the heat stress due to a network of ions which covered the effected parts of plants (Ladha et al. 2005 ; Cassman et al. 2002). The injured cell showed a deprived function during the stress periods. The timely application of nutrients makes their role positive in stress periods (Liu et al., 2011). Literature search revealed that the crops condition under heat stress while but minute work is reported on the flowering crops (Ladha et al. 2005 ; Cassman et al. 2002 ; Dobermann, 2007 ; Rehman, 2016 ; Lima et al. 2015).

Cumulative respirational changes and changes process of marigold flower under heat stress conditions

Several trials on respirational changes were recorded under heat stress conditions (Ladha et al. (2005) Cassman et al. (2002) Dobermann (2007), Rehman, (2016). Lima et al. (2015). Statistical analysis regarding the physiological parameters showed significant differences at $P > 0.05$ for treatments during the year 2015-16 shown in fig 2. The results showed that the maximum respirational rates were recorded in control while lower values were obtained in N_2 0.6 g which was slow at next dose than the increase in turn at P application. These results are according to the findings of Hussein *et al.* (2011) who reported significant role of respiration on growth and yield of marigold under N and P application. The improvement in the photosynthetic pigments compounds which enhanced the production of dry matter, seems to be a valuable tool to interpret the relationship between photosynthesis, respiration, and dry matter production, and, therefore, quality and its yield improves (Hussein *et al.* (2011). Growth efficiency may be associated with the morphology of plants as well as with the chemical nature of respiration which is governed by the environmental conditions and genetic modification and heat tolerance system (Jain *et al.*, 2001).

New Potential nitrogen mineralization (PNM) model of media and Organic residues of media responses under heat stress changes in marigold

The results indicated that the zero-order kinetics model were used to simulate the cumulative mineralized formed of N ($NH_4 + -N$ and TSN) in the laboratory and waterlogged incubation was measured in marigold. The complete model of PNM shown in Table 5. The higher value of $NH_4^+ N$ ($mg\ kg^{-1}$) 190 was found in 1 g of P application, $NH_3^+ N$ ($mg\ Kg^{-1}$) 2.22 was found in same doze of P application. The net uptake of $N\ kg.g^{-1}$ 4.20 was reordered in application of 0.9 N similar in organic matters and C:N were found here in application of 1g p and higher doses of N. The higher value of N org (media) and N org (media) + min 4.1 and 5.5 was measured in application of N 0.9 g per pot shown in table 6. The lower values of N in org and media response were lower due to addition nitrogen was less in media and residue based changes were found as reported by Araus in his experiment. The mineral response in media was changed to less update of N while this critical periods were the N its update is major response of growth in flowers Lima et al. 2015, Rehman, 2016.).

Conclusion

Exposure the plant parts for prolonged heat stress periods has negative effects on phytochemical, physiological process of calendula under critical months of summer in Asia regions. The study was prove the application of Nitrogen and Phosphorus fertilizers play an effective role in minimising the damages during summer seasons periods as supplementary dozes apply on their active growth and developmental stages. The new and advanced biotechnology systematic changes were held in this study as new science of era for safely of florets. The application of Nitrogen 1 g showed a potential improvement of Systematic prompting the new physiological and morphological, biological responses with a positive aspect on phytochemical and physiological improvement by Phosphorus apply at 1 g. The foliar sprays of N and P has potential effects on physiological changes and tagger the response of growth and developmental process at active periods by the timely application of nutritional dozes at stress periods and proved the NUE and PUE was best method for plant health response's The efficacy of Nitrogen and Phosphorus is clear indicator of plants observed nutrients at critical response.

Declarations

Ethics declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The other authors declare that they have no competing interests.

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Authors' Contributions

WA contributed in collecting plant sample, identification and herbarium confection. Conceived and designer the experiments: WA, RA. Performed the experiments RA, AQ,AM, ML. Analyzed the data: WA,AQ,MA,AM,SMK. Wrote the paper: WA, RA, ML, and SMK. All the authors have read the final manuscript and approved the submission.

Compliance with ethical standards

No potential conflict of interest was reported by the authors.

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Tables

Table 1 :Physicochemical properties and active ingredient of media used for seeds and roots culture of marigold flower

Properties	Media (Cocopeat + Sand) for seeds)	Media (Cocopeat+ Sand+ Mould) for root
pH	7.02	6.90
Available P (mg/Kg)	25.0	59.0
Total N (%)	2.8	5.3
Organic Carbon (%)	2.0	8.0

Table 2: Mean comparison of the effect of different level of N and P on Plant height, Total number of leaves, fresh weight, leaf length, number of branches, and number of flowers of marigold

Fertilizers	Plant height (cm)	Total number of leaves	Fresh weight (g)	Leaf length (cm)	Number of branches	Number of flowers
Control	4.95d	10.55 e	18.45e	5.4d	3.66e	3.22d
N 0.6 g per pot	8.47b	14.44b	70.24b	7.86b	7.1b	4.88c
N 0.9 g per pot	8.60 a	15.44a	72.24 a	9.86a	8.1a	5.22b
P 0.8 g per pot	7.17c	13.22d	59.81d	7.2cb	4.66c	4.66c
P 1 g per pot	7.70cb	14.22 c	60.82c	7.1bc	3.22d	5.88a
LSD = 0.012						

Note: Different letters in superscript within the same row indicate significant difference among different harvesting dates at $p < 0.05$ by LSD test. 0.011

Table 3: Mean efficacy of various levels of N and P on Malondialdehyde (MDA) contents, Chrophyll contents, Lipid Peroxidation Assay in leaves, Li and K contents of pot marigold during stress months

Months of Heat stress	Treatments	Malondialdehyde (MDA) contents per gram FW	Chrophyll contents in leaves (%)	Lipid Peroxidation Assay per gram FW	Li and K contents Flux membrane stability (mg dm-3)
May	Control	120e	20e	14b	30e
	N 0.6 g per pot	125d	40b	8e	50d
	N 0.9 g per pot	130c	42a	9d	55c
	P 0.8 g per pot	140b	35d	12c	60b
	P 1 g per pot	150a	38c	15a	65a
June	Control	110ef	18e	13b	25f
	N 0.6 g per pot	130d	39b	7e	60de
	N 0.9 g per pot	135c	41a	10d	70a
	P 0.8 g per pot	141b	34d	11c	64cd
	P 1 g per pot	155a	37c	16a	68b
July	Control	105f	18e	10e	24ef
	N 0.6 g per pot	140e	39b	12d	50d
	N 0.9 g per pot	149cd	41a	18a	72a
	P 0.8 g per pot	155b	34d	14c	60c
	P 1 g per pot	158a	37c	17b	69b
LSD = 0.011					

Note: Different letters in superscript within the same row indicate significant difference among different harvesting dates at $p < 0.05$ by LSD test.

Table 4: Means comparison of various levels of N and P on Phytochemicals Changes of Marigold flowers

Years	2015				2016			
Treatments	Phytochemicals of marigold with two growing years							
	TPC (mg GAE/g)	TA (%DPPH)	TC (mg/100g)	TFC (mg/100g)	TPC (mg GAE/g)	TA (%DPPH)	TC (mg/100g)	TFC (mg/100g)
Control	50.32 e	30.3 e	12.1 e	0.51 e	49.32 e	30.3 e	11.1 e	0.51 e
N 0.6 g per pot	65.55d	34.31b	13.3 d	0.71 c	65.55d	34.31b	13.3 d	0.71 c
N 0.9 g per pot	84.41 a	36.3c a	16.2 a	0.90a	84.41 a	36.3c a	16.2 a	0.90a
P 0.8 g per pot	71.22 c	33.1 c	15.2 b	0.81b	71.22 c	32.1 c	15.2 b	0.81b
P 1 g per pot	83.21 b	32.2d	14.1 c	0.70 d	82.21 b	31.2d	13.1 c	0.70 d
LSD = 0.01								

Table 5 : advanced modern model of Organic residues of media responses

Treatments	NH ₄ + N (mg kg ⁻¹)	NH ₃ + N (mg Kg ⁻¹)	N kg.g ⁻¹	Organic matter g	C:N
Control	70	0.40	2.0	220	10.2
N 0.6 g per pot	110	1.1	4.0	270	11.1
N 0.9 g per pot	170	1.25	4.20	310	12.4
P 0.8 g per pot	180	2.1	2.20	320	20.2
P 1 g per pot	190	2.22	2.21	330	22.2

Table 6 New and active Potential nitrogen mineralization (PNM) of media calculations methods

Treatments	N org (media)	N org (media) + min
Control	2.1d	1.1e
N 0.6 g per pot	2.2c	2.6d
N 0.9 g per pot	4.1a	5.5a
P 0.8 g per pot	2.2c	3.3c
P 1 g per pot	2.6b	4.1b

Different letters in superscript within the same row indicate significant difference among different harvesting dates at $p<0.05$ by LSD test.

Figures

Figure 1

Effects of Nitrogen and Phosphorus foliar application on number of petals and Flower size of marigold

Figure 2

Effects of N & P on Respirational changes of marigold

Figure 3

Dry weight of flower and Dry weight of leaves

Figure 4

Figure 5
Calculation of N and P efficiency rates before and after sowing of marigold

Figure 6

Net N and P efficiency rates measured on heat stress period in months of (May, June, and July)

Figure 7

New model of Net N and P efficiency rates measured on heat stress period in months of (May, June, and July)