Research Article

Effect of gibberellic acid on growth and flowering attributes of African marigold (Tagetes erecta) in inner terai of Nepal

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ABSTRACT
A field experiment was conducted at Bangaun, Lamahi-3, Dang, Nepal to study the effect of GA3 on growth and flowering attributes of African marigold (Tagetes erecta) in Inner Terai of Nepal. The experiment consists of three replications and 8 treatments and laid out in a randomized complete block design- consisting of various concentrations of GA3 viz. 0ppm, 50ppm, 100ppm, 150ppm, 200ppm, 250ppm, 300ppm, and 350ppm. Kolkata local variety of African marigold was tested. The study revealed that among different concentrations of GA3, 300ppm showed the tallest plant height (72.93cm) and the highest basal diameter (1.49cm). Maximum numbers of primary branches (3.11) and the greatest plant spread (32.11cm) were obtained from 250ppm; similarly, maximum numbers of secondary branches (13.80) were recorded in 350ppm. In the case of floral parameters both 100ppm and 350ppm recorded earlier days to 50% flowering (44.00 days each), days for 100% flowering was recorded almost similar in every treatment that sticks around 54 and 55 days, maximum diameter (5.370cm) of flowers were obtained from 50ppm, the greatest fresh weight (6.180g) was recorded in 350ppm, 250ppm showed a maximum number of flower per plant (104.13), similarly, a longer duration of flowering (58 days) was recorded in 300ppm. Among all treatments, the 250ppm level of GA3 was found to be most suitable in terms of production perspective.

Keywords: Concentration, Flowering, GA3, Growth, Merigold

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INTRODUCTION
African marigold (Tagetes erecta) belonging to the Compositae family is one of the important flower crops grown in Nepal. Marigold is a potential commercial flower with growing demand in the context of Nepal due to its cultural and religious importance (Adhikari et al., 2020). It is the tallest of all the species reaching up to three to four feet in height. Easy culture, wider adaptability, the habit of free flowering, short duration to produce marketable flowers and lucrative returns are the reasons behind its increasing popularity. A wide range of colors, shape, size, and good keeping quality, makes the flower more popular (Kumar et al., 2010). In the South Asian region marigold is used as a loose flower for decorations, preparation of garlands, and also for landscaping, religious, and social purposes (Kumar et al., 2010). Extract of Marigold can be used as a nematicide (Ravindra et al., 2017; Marahatta et al., 2012). Carotenoids extracted from dry petals are used for poultry feeds to improve egg yolk color and
Broiler’s skin (Singh, 2014; Singh & Sisodia, 2017). They are also used as a trap crop for controlling different insects like tomato fruit borers. Oil extracted from marigold is used in manufacturing perfumes and insect repellents. Propagation of this flower can be done by using seeds or by softwood cutting. The cutting method is commonly followed for maintaining the purity of varieties.

Plant growth regulators play a significant role in vegetative propagation, inhibition of abscission, prevention of bud dormancy, growth control, and promotion of flowering, prolonging the vase life of flowers, and retarding senescence (Singh et al., 2018). Exogenous foliar application of growth regulator stimulates pollination, fertilization, and seed set to get maximum yield (Dodd goudar et al., 2002). GA3 helps in improving the quality of the flower and is used to overcome the growth limiting factors to harness maximum benefit. It also helps to promote plant growth, an increased number of primary and secondary branches, and also supposed to increase flower quality and maintains uniformity in flower size and number which eventually ensures higher production of flowers. Both higher and lower concentrations of exogenously applied GA3 reduce the vegetative, flowering, and quality parameters as GA3 worked with its full potential up to certain optimum concentration and feedback inhibition occurred beyond such concentrations. Therefore, this study was undertaken with the objective to get a standardized concentration of GA3 on the growth and flowering of African marigold in the Inner Terai of Nepal.

MATERIALS AND METHODS

Experimental site
The experiment with the objective to assess the effect of gibberellic acid on growth and flowering attributes of African marigold was conducted in farmers field at Bangaun, Lamahi - 3, Dang which is located in inner terai of Nepal from August 2019 to December 2019. Geographically, it is located at 27°52′12″N latitude and 82°33′16″E longitude at an elevation of 302 masl having humid subtropical climate. The average temperature during the whole experiment was 22.5°C. The soil of the site was slightly alkaline in pH (7.2), high in organic matter (4.5%) and available phosphorus (71 kg/ha) and medium level of total Nitrogen (0.2%) and potassium (218 kg/ha).

Experimental design and treatment details
The experiment was laid out in a single factorial randomized complete block design with 3 replications and 8 treatments having a total area of 214.5 sq. meter (19.5m x11m) and that of each plot was 6 sq. meter (2m x 3m). The treatments were randomly allocated by using random number table.

Table 1- List of treatment used in research

| Treatments | Concentration of GA3 (ppm) |
|------------|---------------------------|
| T1         | 0                         |
| T2         | 50                        |
| T3         | 100                       |
| T4         | 150                       |
| T5         | 200                       |
| T6         | 250                       |
| T7         | 300                       |
| T8         | 350                       |
The spacing between replication was 1m and between plots was 0.5m. There were 5 rows and 5 columns in each plot. Each plot has 25 plants and total plant population in the field was 600 with plant geometry of 60cm x 40cm.

**Experimental material and cultivation practices**

Marigold cuttings of variety Kolkata local were supplied by Floriculture Cooperative Limited Joytinagar, Chitwan and Plant growth hormone called gibberellic acid (GA3) was brought from BTC Private Limited Kupondol, Lalitpur. Field was prepared by Primary tillage by disk plough (15 days prior to transplanting) and Secondary tillage by power tiller. Fifteen days old, healthy and uniform marigold cuttings were transplanted on 29th August 2019. Irrigation was applied just after transplanting and successively on weekly basis. FYM was applied @ 10 t/ha during primary tillage and N:P: K was applied @ 200:80:80 kg/ha (FAN, 2016) where Nitrogen was split into two equal doses. Half dose of N and full dose of P, K was applied as basal dose and remaining half dose of N was applied at 20 DAT. Manual weeding and earthing up was performed at 20 and 40 days after transplanting. Pinching was performed at 25 DAT by removing top 3-4 cm of plants. Chemical method of pest control was applied during the experiment for the sake of controlling insect and diseases. Harvesting of flower was done three times at 55, 73 and 93 DAT as flowering in marigold is not synchronizing. It was performed manually by plucking the flower in the morning after dew had been dried up. Harvesting was done when the central whorls of petals were fully open. Irrigating the field, a day before each harvest was mandatorily followed to improve shelf life of cut flower.

**Formulation and application of GA3**

GA3 hormone available with trade name GIBBERELIC ACID RM9157-1G @ 1 g per glass was in powder form that has been stored to temperature less than 2 degree Celsius. Seven different concentrations viz., 50, 100, 150, 200, 250, 300 and 350 ppm of gibberellic acid (GA3) were prepared just before their application. GA3 was weighed with the help of digital weighing balance having capacity of measuring ranges from 0.01g to 500g. For preparing 50 ppm of GA3 solution 0.05g of GA3 was dissolved in 1 litre of water. With same procedure other solution of different concentration of GA3 was prepared. Foliar application of GA3 was done at 25 DAT by using hand atomizer at evening time after pinching. While in control treatment water was sprayed at the same time.

**Observation recorded**

Observation on vegetative attributes including plant height, number of primary and secondary branches and basal diameter were made. Under phenological parameter days to 50% and 100% flowering and duration of flowering were observed. Similarly, the observed yield parameter was number of flowers per plant, fresh weight of flower and diameter of flower. All observation was made from the 5 randomly selected plants of each plot.

**Data Management and Analysis**

The collected data were entered, tabulated and processed in Microsoft Excel 2016. Data were analysed through GenStat 18th edition statistical package. Means were separated by least significant difference (LSD) test at 1% or 5% level of significance (Gomez & Gomez 1984; Shrestha, 2019) and Duncan’s Multiple Range Test (DMRT) at 5% level of significance. Pearson’s correlation coefficient and regression equation were run between selected parameters wherever necessary.
RESULTS AND DISCUSSION

Growth parameters

Plant height
The application of GA3 significantly (P<0.05) affected the plant height of marigold measured at harvest. Significantly higher plant height was recorded in a higher concentration of GA3 than control at harvest. Tallest plant (72.93cm) was recorded in flowers applied with 300ppm GA3 in comparison with those applied with 100ppm GA3 (62.13cm) and grown in controlled condition (64.77cm) but was at par with the ones applied with 250ppm GA3 (72.00cm) and 350ppm GA3 (71.93cm). Thus, it showed that plant height increased with an increase in GA3 concentrations.

An experiment conducted by Sarkar et al. (2018) to study the ‘response of Pinching and Gibberellic Acid on Growth and Physiological Characteristics of African Marigold’ resulted in similar findings. Owing to the fact increased in GA3 application increased the intermodal length and cell enlargement that increases growth of plants and also increases auxin content which enhanced the apical dominance indirectly. The results obtained were concordance with the findings of Taygi and Kumar (2006), Kumar et al. (2010) and Badge et al. (2013) in marigold.

Plant spread
Level of GA3on plant spread was found no significant (p>0.05). However, the highest plant spread (32.11cm) was obtained from plants applied with 250ppm GA3 whereas as lowest plant spread (26.01cm) was obtained from 200ppm GA3 treatment. Our findings show increase plant spread with application of GA3 which was supported by result obtained from (Gautam et al., 2006). This may be resulted due to extension in plant height and increased in main axis count caused by hyper elongation of inter nodal length. Optimum plant spread resulted from increased primary branches which are originated from dormant bud with increase in main axis count. This fluctuation in value of plant spread may be due to external factors such as climatic fluctuation, insects, nutrient conditions in soil and diseases.

Basal diameter
The research revealed that basal diameter was insignificant to application of different doses of GA3. However, the highest basal diameter (1.49cm) was obtained from 300ppm GA3 treatment and the lowest (1.257cm) from control. The data showed increased basal diameter on increasing the doses of GA3 up to 300ppm and decreased beyond this concentration. The result was in conformity with the finding of Khangjarakpam et al. (2019) who reported higher doses of GA3 decreases basal diameter due to inhibitory action of GA3 on cell division and elongation as GA3 shows feedback inhibition after optimum concentration.

Number of primary branches
The experiment revealed that there was no any significant difference in number of primary branches due to the application of different level of GA3. However, highest primary branches (3.133) were obtained in treatment T6 (250ppm GA3) and lowest (2.8) in treatment T1 (control). The data presented on table show a little increment in number of primary branches with higher concentration of GA3.
The reason behind the insignificant result on number of primary branches may be due to the use of cutting as a planting material. Cell differentiation that directs the formation of primary branches might have already occurred before application of GA3 in cuttings so it may not have produced significant difference in number of primary branches.

**Number of secondary branches**

Numbers of secondary branches per plants were significantly differed with the application of different level of GA3 solution. Significantly highest number of secondary branches (13.80) was recorded in 350ppm GA3 which was at par with GA3300ppm (12.97), GA3250ppm (13.67) and GA3150ppm (12.23). Lowest number of secondary branches (10.27) was observed in control treatment.

Thus, from obtained data we can conclude that just a stimulation of GA3 can able to produce significantly higher number of secondary branches. As an application of GA3 enhanced cell division and cell enlargement, promotion of protein synthesis and stimulation of branching may be attributed to the removal of apical dominance by the pinching we performed. Results obtained were in accordance with the findings of Singh and Arora (1980) on African marigold; Kumar and Singh (2003) in carnation, Srivastava et al. (2002) and Lal and Mishra (1986) in China aster and marigold.

**Table 2. Effect of different doses of GA3 in growth parameters of marigold in Lamahi-4 Deukhuri, Dang, Nepal (2019)**

| Treatments | Plant height (cm) | Plant spread (cm) | Basal diameter (cm) | No. of primary branches | No. of secondary branches |
|------------|------------------|-------------------|---------------------|------------------------|--------------------------|
| Control    | 62.13c           | 27.96ab           | 1.257               | 2.800                  | 10.27c                   |
| 50ppm GA3  | 64.77bc          | 29.28ab           | 1.357               | 2.900                  | 11.87bcde                |
| 100ppm GA3 | 68.73ab          | 26.98ab           | 1.273               | 2.933                  | 11.00de                  |
| 150ppm GA3 | 68.33abc         | 31.32a            | 1.320               | 2.967                  | 12.23abcd                |
| 200ppm GA3 | 69.73ab          | 26.01b            | 1.340               | 2.833                  | 11.50cde                 |
| 250ppm GA3 | 72.00a           | 32.11a            | 1.390               | 3.133                  | 13.67ab                  |
| 300ppm GA3 | 72.93a           | 27.88ab           | 1.493               | 3.000                  | 12.97abc                 |
| 350ppm GA3 | 71.93a           | 27.37ab           | 1.267               | 3.067                  | 13.80a                   |
| Grand mean | 68.82            | 28.61             | 1.337               | 2.954                  | 12.16                    |

Variance estimation:

- CV (%) 5.0 9.3 10.9 8.6 8.2
- SEM (±) 2.225 2.168 0.119 0.207 0.817
- LSD (0.05) 6.072 4.649 0.2554 0.4459 1.753
- F Test NS NS NS NS NS

Treatments means followed by the common letter (s) within column are non-significantly different among each other based on DMRT at 5% level of significance. LSD = Least significant difference, NS = Non Significant, * denotes significant result and CV = Coefficient of variation.

**Yield and yield attributing characteristics**

**Days to 50% flowering**

The research revealed that Days to 50% flowering was insignificant to the application of different doses of GA3. Days to 50% flowering was recorded earlier in T3 (100ppm) and T8 (350ppm) as compared to T5 (200ppm). This fluctuation in Days to 50% flowering may be due to external factors such as climatic fluctuation, insects, and diseases.
Days to 100% flowering
Although GA3 induces earlier flowering in long day plants like in tomato but in case of this experiment there was no any significance difference in days to 100% flowering by the application of different level of GA3 solution. Every treatment had almost similar days for 100% flowering that stick around 54 and 55 days. This insignificant result on days to 100% flowering may be due to use of cuttings as a planting material as they are already physiologically capable of giving flower earlier so external application of GA3 may not be able to make much earlier flowering. An experiment conducted in chrysanthemum by Valeru et al. (2018) recorded the minor differences in buttoning and flower opening which might be due to strong influence of pre availed short day conditions. As marigold is also a short day plants similar conditions may applied.

Diameter of flower
Experiment revealed that the different levels of GA3 were not differed significantly in diameter of flower. However highest flower diameter (5.37cm) was obtained in treatment T2 (500ppm GA3) and lowest (4.93cm) was obtained in treatment T1 (control). The data showed that with application of GA3 solution, diameter of flower was increased but not in the linear pattern, which was supported by results obtained from Pandey et al. (2015). A result obtained was in compliance with the findings of Dalal et al. (2009). Dry matter accumulation in plant due to cell division, cell enlargement and protein synthesis caused increment in flower weight. Non-linear increment in the flower diameter with application of different level of GA3 might be also due to climatic condition, insect pest, disease (blight) and non-uniform nutrient content in the soil.

Fresh weight of flower
Gibberellic acid significantly (P<0.05) affected the fresh weight of flowers at harvest. Significantly greater fresh weight was recorded in a higher concentration of GA3 than control at harvest. Significantly greater fresh weight was recorded in 350 ppm of GA3 (6.180g) in comparison with GA3 50 ppm (5.110g) and control treatment (5.190g) but was at par with GA3 200 ppm (5.707g), 250 ppm (5.737g) and 300 ppm (5.787g). Thus, it was found that fresh flower weight increased with an increase in GA3 concentrations. Stimulation of the corella growth, pollen germination, and pollen tube growth occurred with the GA3 application which in turn increases weight of flower. Similar results were recorded by Kumar et al., 2010; Ardalani et al., 2014; Kumar and Beniwal, 2017; Tiwari H. 2018; Sarkar D. 2018 in marigold and Holkar P.S. 2018 in Gladiolus.

No. of flower
Levels of gibberellic acid were differed significantly in number of flower per plant of marigold. Significantly higher number of flowers per plant (104.13) was recorded from treatment 250 ppm GA3 and the lowest (70.6) from treatment control. Our research finding showed plant applied with GA3 250 ppm was found to be effective to produce maximum number of flowers per plants. Similar result was observed in experimentation done by Khangjarkpam et al., (2019) in African marigold.

Increased number of flower when treated with 250 ppm GA3 was because this treatment resulted in maximum chlorophyll content and protein content in leaf and had stimulatory role to
decrease the activity of chlorophyllase enzymes thus prevents chlorophyll and protein degradation leading to enhancement of rate of photosynthesis. Under the control of GA3, partitioning of photosynthates to reproductive sink occurred which resulted in maximum number of flowers per plant (Morris, 1996).

**Duration of flowering**

The research revealed that duration of flower was differed significantly \((P<0.05)\). Significantly longer duration of flowering (58 days) was recorded in 300ppm GA3 treatment in comparison to shorter duration of flowering (54.67 days) which was recorded in 50ppm but was at par with GA3350ppm (57.67 days) and GA3150ppm (57.00 days).

Maximum duration of flowering with treatment 7 (300ppm GA3) was probably due to reduction in juvenile period in the interphase of cell cycle as reduction of S-phase promote the shoot apical meristem to starts producing buds instead of producing leaves and branches. (Khangjarakpam et al., 2019). Similar findings were obtained by Kumar et al. (2010) and were also observed by Nair et al. (2002) in gerbera.

**Yield per plant**

Level of gibberellic acid was differed highly significant in yield of marigold. However highest flower yield per plant (0.5980 kg) was observed in 250ppm GA3 which was at par with 300ppm GA3 (0.5847 kg) and 350ppm GA3 (0.54 kg) and lowest yield per plant (0.3630 kg) was observed in control. The results obtained were in accord with findings of Khangjarakpam et al., (2019) who reported highest flower yield recorded in plants sprayed with GA3 250ppm perhaps probably as account of production of flower with increased flower weight and greater diameter. Greater diameter of flower which in turns induced through high number of florets as a result of better nutrition during reproductive phase.

**Table 3 Effect of different doses of GA3 in yield and yield attributes of marigold in Lamahi-4 Deukhuri, Dang, Nepal (2019)**

| Treatments | Days to 50% flowering (days) | Days to 100% flowering (days) | Diameter of flower (cm) | Fresh weight of flower (g) | No. of flower | Duration of flowering (days) | Yield per plant (kg) |
|------------|------------------------------|------------------------------|-------------------------|----------------------------|--------------|-------------------------------|---------------------|
| Control    | 46.67ab                      | 54.00                        | 4.937                   | 5.190c                     | 70.06d       | 55.33bc                       | 0.3630d             |
| 50ppm GA3  | 44.67ab                      | 54.00                        | 5.370                   | 5.110c                     | 90.11bc      | 54.67c                        | 0.4600c             |
| 100ppm GA3 | 44.00b                       | 54.67                        | 5.287                   | 5.380bc                    | 85.49c       | 56.00abc                      | 0.4587c             |
| 150ppm GA3 | 47.33ab                      | 55.33                        | 5.163                   | 5.410bc                    | 92.11bc      | 57.00ab                       | 0.4977bc            |
| 200ppm GA3 | 47.33ab                      | 55.33                        | 5.163                   | 5.707ab                    | 85.43c       | 56.33abc                      | 0.4867bc            |
| 250ppm GA3 | 45.33ab                      | 55.00                        | 5.120                   | 5.737ab                    | 104.13a      | 56.67abc                      | 0.5980a             |
| 300ppm GA3 | 45.33ab                      | 54.67                        | 5.147                   | 5.787ab                    | 101.07ab     | 58.00a                        | 0.5847a             |
| 350ppm GA3 | 44.00b                       | 55.33                        | 5.257                   | 6.180a                     | 87.60c       | 57.67a                        | 0.5400ab            |
| Grand mean | 45.33                        | 54.71                        | 5.196                   | 5.562                      | 89.5         | 56.46                          | 0.4986              |
| CV (%)     | 3.3                          | 1.6                          | 7.5                     | 4.8                        | 7.2          | 1.8                           | 8.2                 |
| SEM (±)    | 1.208                        | 0.699                        | 0.31                    | 0.220                      | 5.26         | 0.852                         | 0.033               |
| LSD (0.05) | 2.592                        | 1.498                        | 0.6801                  | 0.4723                     | 11.28        | 1.828                         | 0.07127             |
| F Test     | 0.143                        | NS                           | NS                      | **                         | ***          | *                             | **                  |

*Treatments means followed by the common letter (s) within column are non-significantly different among each other based on DMRT at 5% level of significance. LSD = Least significant difference, NS = Non Significant, * denotes significant result and CV = Coefficient of variation.*
Correlation and regression

A positive correlation was observed between plant height and yield per plant (Figure 1). Plant height at harvest and flower yield per plant is strongly linear and positively related meaning that as the plant height increases, the flower yield per plant increases. The coefficient of determination 0.40 signifies that contribution of plant height at harvest on flower yield per plant is 40% and the rest of the effect was due to other factors.

![Figure 1: Relationship between plant height at harvest and flower yield per plant of marigold flower in Lamahi-4, Dang, Nepal (2019).](image1)

\[ y = 0.0112x - 0.2768 \]
\[ R^2 = 0.4008 \]

A highly significant positive correlation was observed between number of secondary branches and yield of marigold flower. Number of secondary branches and flower yield is linearly and positively related meaning that as the no. of secondary branches increases, the flower yield also increases. The coefficient of determination (R²) value is 0.230. It was observed that no. of secondary branches contributes about 23% change in flower yield (R²=0.230) whereas, rest of the change arises due to other factors.

![Figure 2: Relationship between secondary branches and flower yield per plant of marigold flower in Lamahi-4, Dang, Nepal (2019).](image2)

\[ y = 0.0194x + 0.2631 \]
\[ R^2 = 0.2306 \]
A positive correlation was observed between fresh flower weight and yield per plant (Figure 3). Fresh flower weight at harvest and flower yield per plant is strongly linear and positively related which means as the fresh flower weight increases, the flower yield per plant increases. The coefficient of determination value ($R^2$) is 0.412. It implies, fresh flower weight contributes 41.2% on flower yield per plant and rest was due to other factors.

![Figure 3: Relationship between flower weight and flower yield per plant of marigold flower in Lamahi-4, Dang, Nepal (2019).](image)

A significant positive correlation was observed between Number of flower and yield of marigold flower (Figure 4). Number of flower and flower yield is linearly and positively related that means as the number of flower increases, the flower yield also increases. The coefficient of determination ($R^2$) 0.826, reveals that, the contribution of number of flower on flower yield is 82.6% and rest of the effect was due to other factors.

![Figure 4: Relationship between number of flower and flower yield per plant of marigold flower in Lamahi-4, Dang, Nepal (2019).](image)

A significant positive correlation was observed between duration of flowering and yield of marigold flower (Figure 5). Duration of flowering and flower yield is linearly and positively related meaning that as the duration of flowering increases, the flower yield also increases. The coefficient of determination ($R^2$) 0.350, discloses that the contribution of duration of flowering on flower yield is 35% and rest of the effect was due to other factors.
Figure 5: Relationship between duration of flowering and flower yield per plant of marigold flower in Lamahi-4, Dang, Nepal (2019).

CONCLUSION
From the above result, the effect of gibberellic acid was mainly seen on plant height, number of secondary branches, fresh weight of flower, number of flowers per plant, duration of flowering and flower yield per plant while other parameter remain statistically insensitive to the application of GA3. From all these findings we can conclude that yield of marigold flower can be increase to a significant level with the application of GA3of particular concentration. In this experiment among all treatments GA3250ppm (T6) was found to be most suitable to in terms of production. On the basis of this experiment, we highly suggest commercial farmer of marigold around inner terai of Nepal to spray 250ppm of GA3at 25 DAT.

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Authors’ contributions
B. Ghimire, S. Acharya and S. Gaihre designed and performed experiment, recorded data, analyzed data and wrote the paper. K. Aryal and L.B Chhetri supervised the experiment and edited the paper.

Conflict of Interest
The authors declare no conflict of interest regarding the publication of this manuscript.

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