

**Original Research Paper**

## Effect of nano and macro iron sprays on growth, flowering, seed and oil yielding attributes in calendula (*Calendula officinalis* L.)

Srivastava R.K.<sup>1</sup>, Tarakeshwari K.R.<sup>1</sup>, Bhandari N.S.<sup>2\*</sup> and Chand S.<sup>1</sup>

<sup>1</sup>Department of Horticulture, G.B. Pant University of Agriculture & Technology, Pant Nagar, U.S. Nagar, Uttarakhand, India

<sup>2</sup>School of Agriculture, Graphic Era Hill university, Bhimtal Campus, Nainital, Uttarakhand, India

\*Corresponding author Email : nsbhandari@gehu.ac.in

### ABSTRACT

The investigation was executed with nine treatments viz. nano forms of ferrous sulfide (7, 14, 21, 28 ppm) and macro ferrous sulphate (0.2, 0.4, 0.6, 0.8 per cent) along with control, and were applied as foliar sprays after 30 days of transplanting on two varieties of calendula namely 'Fiesta Gitana Mix' and 'Fiesta Yellow' during 2018 and 2019. The experiment was laid out in factorial randomized block design with three replications. Application of 0.8 % FeSO<sub>4</sub> recorded maximum number of branches (26.75), plant height (29.73 cm), plant spread (45.17 cm), number of leaves (22.63) and seed test weight (15.63 g) and number of flowers per plant (134.04). However, application of 0.2% macro FeSO<sub>4</sub> resulted in early bud appearance (50.50 days) and higher flower diameter (8.09 cm). 'Fiesta Gitana Mix' outperformed over 'Fiesta Yellow' for most of the vegetative and floral characters. The 'Fiesta Yellow' variety with oil content (13.97%) had an edge over 'Fiesta Gitana Mix'.

**Key words:** Calendula, Ferrous sulphate, Flowering, Nano iron, Oil content and Seed yield.

### INTRODUCTION

*Calendula officinalis* is a member of the family Asteraceae. It is cultivated in Eastern Europe, West Asia, Germany and USA. It is also known as pot marigold, calendula, ringer blume, *souci des jardins* in different countries (Sahingil, 2019). It is an economic plant for its beautiful flowers, herbal and cosmetic products. It is also used traditionally as culinary and medicinal herb. The petals are edible and can be used afresh in salads or dried and used in coloring cheese or as a replacement for saffron. The petals color varies from yellow to orange and has an aromatic scent (Saffari and Saffari, 2020). A yellow dye has been extracted from the flowers. Skin products from calendula are used to treat minor cuts, burns and skin irritations and other ailments. These various uses are attributed by constituents such as flavanol glycosides, triterpene oligoglycosides, saponins and sesquiterpene glucoside. Flower heads are sources of carotenoids which help for improved vision, normal growth and development and flavanoids which possess anti-viral and anti-cancer properties (Khalid and Teixeira da Silva, 2010). An increasing interest in calendula cultivation has been witnessed in recent

years as an oil-bearing plant whose seeds were reported to contain unique poly unsaturated fatty acids which have the potential to be used in paint, coatings and pharmaceutical industries (Krol and Paszko, 2017).

Nano-fertilizers are currently a novel technology that allows for much more absorption by miniaturization of the particle size in nano scales. High absorbability and consumption both through the soil and the leaves are the characteristics of these types of fertilizers. The slow-releasing property of nano-fertilizers has a major contribution to their optimal use (Alamdari *et al.*, 2021). This enables nano-particles (NPs) to boost the plant's metabolism. Application of nano-fertilizers promoted growth, development, antioxidant activity, stress tolerance and total phenol content (TPC) in many crops with lesser concentration.

Iron NPs due to their nano size as well as magnetic characteristics are considered as special nano-fertilizers. The bio-compatibility as well as interaction between plants and the Fe nano-particles had led to a great deal of attentions. The Fe nano-particles effect plants in two ways, lower concentrations of FeNPs had positive effects on the growth and physiology of crop



plants, whereas, high concentrations had toxic effects on plants. Fe nano-particles were reported with nutrient absorption promotion as well as photosynthetic efficiency enhancement. The use of nanotechnological inventions in calendula production having potential as landscaping, ornamental as well as medicinal plant can prove as beneficial research environmentally, economically and aesthetically. Therefore, the present investigation was planned to assess the impact of different concentrations of nano and macro forms of iron on growth, flowering, seed yield and oil content in *C. officinalis*.

## MATERIALS AND METHODS

The experimental site was situated in the *Tarai* region of Uttarakhand, India at 29° N latitude and 79.3° E longitudes in the foot hills of the Himalaya at an altitude of 243.84 m above mean sea level. The soils of the experimental field were sandy loam having pH 6.68, organic carbon (0.60%), available N, P and K as 231.91, 18.34 and 135.97 Kg ha<sup>-1</sup>, respectively. Well rotten farmyard manure @ 5 kg/m<sup>2</sup> was incorporated into soil at the time of bed preparation. Calendula seeds of two varieties namely “Fiesta Gitana Mix” and “Fiesta Yellow” were sown in well prepared nursery beds. Upon germination, 25-day-old seedlings were transplanted in the experimental field at a spacing of 60 cm × 30 cm. The experiment was conducted in factorial randomized block design with nine treatments replicated thrice. Five plants per treatment per replication were randomly selected for observations.

### Nano and macro iron treatments

For nano-iron treatments, a stock solution of 28 ppm nano FeS was diluted with distilled water to make four different concentrations (7, 14, 21 and 28 ppm). For ferrous sulphate solution, different quantities (2, 4, 6 and 8g) of FeSO<sub>4</sub> salt were dissolved separately in 1000 ml of slaked lime water to prepare solutions of required concentrations. Nano-iron (iron sulfide) solutions of 7, 14, 21 and 28 ppm and ferrous sulphate solutions of 0.2, 0.4, 0.6 and 0.8 per cent concentrations were sprayed 30 days after transplanting. All other cultural conditions such as hoeing, weeding, irrigation, etc were kept uniform for all the treatments.

### Oil extraction from seeds

The oil from seeds of calendula was extracted using solvent extraction method. Soxhlet apparatus was used

for extraction using hexane as a solvent. The pooled data for both the years 2018 and 2019 were statistically analyzed using the software ‘OPSTAT’(8).

## RESULTS AND DISCUSSION

Data presented in Table 1 indicated that vegetative traits such as number of branches, plant height, plant spread and number of leaves significantly affected by treatments, varieties and their interaction.

Irrespective of the varieties, spray of FeSO<sub>4</sub> recorded significantly more number of branches (26.75) in T<sub>9</sub> than rest of the treatments, however, it was recorded minimum (14.50) in control (T<sub>1</sub>). The variety Fiesta Yellow had maximum number of branches (20.07) over variety Fiesta Gitana Mix (18.45). Among the interaction, a greater number of branches (29.58) were recorded in V<sub>1</sub>T<sub>9</sub> followed by V<sub>2</sub>T<sub>6</sub> (25.17) which were statistically at par with other treatments. However, number of branches was recorded minimum (12.00) in variety Fiesta Gitana Mix sprayed with 21 ppm nano FeS (V<sub>1</sub>T<sub>2</sub>). Torabian *et al.* (2018) reported that increased growth in sunflower grown under saline condition by application of FeSO<sub>4</sub> both in normal and nano form which is due to increased leaf area, net CO<sub>2</sub> assimilation, sub-stomatal CO<sub>2</sub> concentration, chlorophyll content, etc. Likewise, Yuan *et al.* (2018) also reported that iron NPs promoted plant growth of *Capsicum annum* by increasing chloroplast numbers and grana stacking. In the present investigation, the lesser number of branches in nanoparticle treated plants against macro iron treatment might be due to their insufficient quantity as compared to macro forms.

Irrespective of varieties, the maximum plant height was recorded in T<sub>9</sub> (29.73 cm) which was at par with T<sub>8</sub> (29.42 cm) and T<sub>7</sub> (27.86 cm) but significantly higher than the rest of the treatments. However, minimum was recorded in T<sub>2</sub> (21.19 cm). The plant height was significantly higher in variety Fiesta Gitana Mix (26.18 cm) than variety Fiesta Yellow (23.63 cm). Among the interaction, maximum plant height (31.96 cm) was recorded in V<sub>1</sub>T<sub>9</sub> combination followed by V<sub>1</sub>T<sub>8</sub> (30.84), and V<sub>1</sub>T<sub>7</sub> (30.34) and it was minimum (20.54 cm) in V<sub>2</sub>T<sub>2</sub>. Treatments T<sub>9</sub> (31.96 cm) and T<sub>8</sub> (28.00 cm) showed significant effect on plant height over nano iron and control in varieties Fiesta Gitana Mix and Fiesta Yellow, respectively. However, among nano iron treatments, T<sub>5</sub> (24.75cm) and T<sub>4</sub> (22.17 cm) had maximum plant height in variety Fiesta Gitana Mix (V<sub>1</sub>) and Fiesta Yellow (V<sub>2</sub>), respectively. Both NPs treatments were more effective than control but less effective as compared to iron in normal form. The

**Table 1 : Effect of different concentrations of nano and macro forms of iron on vegetative characters in calendula varieties ‘Fiesta Gitana Mix (V<sub>1</sub>)’ and ‘Fiesta Yellow (V<sub>2</sub>)’**

| Treatments                               | No. of branches  |                |       | Plant height (cm) |                |       | Plant spread (cm) |                |       | Number of leaves |                |       |
|--|------------------|----------------|-------|-------------------|----------------|-------|-------------------|----------------|-------|------------------|----------------|-------|
|  | V <sub>1</sub>   | V <sub>2</sub> | Mean  | V <sub>1</sub>    | V <sub>2</sub> | Mean  | V <sub>1</sub>    | V <sub>2</sub> | Mean  | V <sub>1</sub>   | V <sub>2</sub> | Mean  |
| T <sub>1</sub> (Control)                 | 12.75            | 16.25          | 14.50 | 23.09             | 20.88          | 21.98 | 40.71             | 40.04          | 40.38 | 20.08            | 17.83          | 18.96 |
| T <sub>2</sub> (7 ppm nano FeS)          | 12.00            | 17.50          | 14.75 | 21.83             | 20.54          | 21.19 | 40.08             | 41.79          | 40.94 | 18.50            | 17.92          | 18.21 |
| T <sub>3</sub> (14 ppm nano FeS)         | 13.25            | 17.42          | 15.33 | 23.21             | 20.96          | 22.09 | 39.46             | 38.92          | 39.19 | 17.75            | 18.33          | 18.04 |
| T <sub>4</sub> (21 ppm nano FeS)         | 14.25            | 15.42          | 14.83 | 22.17             | 22.17          | 22.17 | 40.33             | 41.38          | 40.86 | 18.50            | 19.25          | 18.88 |
| T <sub>5</sub> (28 ppm nano FeS)         | 13.75            | 17.00          | 15.38 | 24.75             | 21.50          | 23.13 | 41.38             | 37.00          | 39.19 | 22.16            | 17.25          | 19.71 |
| T <sub>6</sub> (0.2% FeSO <sub>4</sub> ) | 21.92            | 25.17          | 23.54 | 27.46             | 25.71          | 26.59 | 41.75             | 44.67          | 43.21 | 26.92            | 17.33          | 22.13 |
| T <sub>7</sub> (0.4% FeSO <sub>4</sub> ) | 23.83            | 24.83          | 24.33 | 30.34             | 25.37          | 27.86 | 43.13             | 42.83          | 42.98 | 23.75            | 19.58          | 21.67 |
| T <sub>8</sub> (0.6% FeSO <sub>4</sub> ) | 24.75            | 23.17          | 23.96 | 30.84             | 28.00          | 29.42 | 44.75             | 43.79          | 44.27 | 26.83            | 19.00          | 22.92 |
| T <sub>9</sub> (0.8% FeSO <sub>4</sub> ) | 29.58            | 23.92          | 26.75 | 31.96             | 27.50          | 29.73 | 45.38             | 44.96          | 45.17 | 25.42            | 19.83          | 22.63 |
| <b>Mean</b>                              | <b>18.45</b>     | <b>20.07</b>   |       | <b>26.18</b>      | <b>23.63</b>   |       | <b>41.88</b>      | <b>41.71</b>   |       | <b>22.21</b>     | <b>18.48</b>   |       |
| <b>Factor</b>                            | <b>C.D. (5%)</b> | <b>SEM</b>     |       | <b>C.D. (5%)</b>  | <b>SEM</b>     |       | <b>C.D. (5%)</b>  | <b>SEM</b>     |       | <b>C.D. (5%)</b> | <b>SEM</b>     |       |
| Variety (V)                              | 0.876            | 0.611          |       | 0.931             | 0.323          |       | 0.67              | 0.48           |       | 1.359            | 0.471          |       |
| Treatments (T)                           | 3.738            | 1.295          |       | 1.976             | 0.684          |       | 2.939             | 1.018          |       | 2.882            | 0.999          |       |
| V×T                                      | 2.015            | 1.832          |       | 1.0436            | 0.968          |       | 1.612             | 1.440          |       | 4.076            | 1.412          |       |

effect of nano iron might be due to increased chlorophyll content which increased photosynthesis, in turn, growth of plants (Ghafari and Razmjoo, 2015). Askary *et al.* (2017) reported increased growth, photosynthetic pigments and total protein contents in peppermint with application of FeO<sub>3</sub> (30µM NPs). Yuan *et al.* (2018) observed low concentration of iron NPs promoted plant growth due to increased chloroplast, number of grana stacking and regulation of vascular bundles. Increase in plant height in Cress was observed by Salarpour *et al.* (2013) upon applying 5g nano iron chelate + foliar spray of iron. Enhanced plant height due foliar spray of iron NPs has been reported (Elfeky *et al.*, 2013).

Irrespective of the varieties, maximum plant spread (45.17cm) was recorded in T<sub>9</sub>, which was statistically at par with T<sub>8</sub> (44.27cm), T<sub>6</sub> (43.21cm) and T<sub>7</sub> (42.98cm) but significantly higher than rest of the treatments. However, minimum plant spread (39.19 cm) was recorded in both, T<sub>3</sub> and T<sub>5</sub>. Maximum spread of plants (41.89 cm) was recorded in variety Fiesta Gitana Mix and minimum in variety Fiesta Yellow (41.71cm). Among the interaction plants' spread was maximum (45.38 cm) in V<sub>1</sub>T<sub>9</sub>, followed by V<sub>2</sub>T<sub>9</sub> (44.96 cm), V<sub>2</sub>T<sub>8</sub> (44.75cm) and V<sub>2</sub>T<sub>6</sub> (44.67 cm). However, least plant spread (37.00 cm) was recorded in V<sub>2</sub>T<sub>5</sub>. Among nanoparticles treatments, T<sub>5</sub> in variety Fiesta Gitana Mix and T<sub>2</sub>-in variety Fiesta Yellow showed higher plant spread over control. Yuan *et al.* (2018) reported improved overall plant growth in capsicum as a result of iron nanoparticles application was due to enhanced chloroplast, grana stacking as well as development of vascular bundles. Pirzad and Shokrani (2012) reported improved plants growth in calendula due to application of iron NPs (1.5 l/ha). In the present investigation, positive influence of NPs for plant spread over control might be due to reduced nutrient loss as reported by Hu *et al.* (2017) in *Citrus maxima* plants.

Irrespective of the varieties, maximum number of leaves (22.92) was recorded in T<sub>8</sub> which was statistically at par with T<sub>6</sub> (22.13) T<sub>7</sub> (21.67) and T<sub>9</sub> (22.63) but significantly higher than rest of the treatments. However, minimum number of leaves (18.04) was recorded in T<sub>3</sub>. The variety Fiesta Gitana Mix recorded maximum number of leaves (22.21) than variety Fiesta Yellow (18.48). Among the interaction, more number of leaves (26.92) was recorded in V<sub>1</sub>T<sub>6</sub> combination followed by V<sub>1</sub>T<sub>8</sub> (26.83) and V<sub>1</sub>T<sub>9</sub>

(25.42) whereas least number of leaves (17.25) was recorded in V<sub>2</sub>T<sub>5</sub>. However, higher dose of nanoparticle resulted in more number of leaves. Praveen *et al.* (2018) reported improved growth of mustard plants treated with NPs (Fe<sub>3</sub>O<sub>4</sub>) mainly due to enhanced availability of iron.

Calendula plants when sprayed with different treatments of iron showed significant response for days to earlier bud appearance, days to bloom, flower diameter and number of flowers per plant (Table 2). The effect of treatments was significant, whereas, varieties and treatments-varieties interactions on days to early bud appearance were non-significant. Among the treatments, irrespective of varieties, maximum days to bud appearance (52.06 days) was recorded in control (T<sub>1</sub>) which was statistically at par with by T<sub>2</sub> (50.92 days) but significantly higher than rest of the treatments. However, minimum number of days to bud appearance (48.63 days) was recorded in T<sub>9</sub>. Tayade *et al.* (2018) reported early initiation of spike in tuberose with 0.4% FeSO<sub>4</sub>.

The days to bloom was significantly influenced by varieties, however, treatment and variety-treatment interaction was non-significant. Significantly more days to bloom (65.00 days) was recorded in variety Fiesta Gitana Mix than variety Fiesta Yellow (64.59 days). Tayade *et al.* (2018) reported early opening of first floret in tuberose with 0.4% FeSO<sub>4</sub>. Goshwami *et al.* (2021) reported that application of 10 ppm of Gold-nanoparticle was found best for number of flowers, flower diameter, flower weight, minimum days to flower bud initiation and flowering duration.

Irrespective of varieties, maximum diameter of flower (8.09 cm) was recorded in T<sub>6</sub> which statistically par with T<sub>3</sub> (7.92 cm) but significantly higher than rest of the treatments, whereas, minimum diameter of flower (7.43 cm) was recorded in T<sub>9</sub>. Barring the treatments, significantly higher flower diameter (7.94 cm) was recorded in variety Fiesta Gitana Mix than variety Fiesta Yellow (7.52 cm). Among interaction maximum diameter of flower (8.33 cm) was recorded for treatment V<sub>1</sub>T<sub>6</sub> and V<sub>1</sub>T<sub>7</sub> and were statistically at par with V<sub>1</sub>T<sub>3</sub> (8.28 cm) V<sub>1</sub>T<sub>5</sub> (8.11 cm) but significantly higher than rest of the interactions. Pirzad and Shokrani (2012) reported that iron NPs @ 1.5 l/ha increased capitulate diameter and in calendula with 0.4% of FeSO<sub>4</sub> (Tayade *et al.*, 2018).

**Table 2 : Effect of different concentrations of nano and macro forms of iron on flowering characters in calendula varieties ‘Fiesta Gitana Mix (V<sub>1</sub>)’ and ‘Fiesta Yellow (V<sub>2</sub>)’**

| Treatments                               | Days to bud appearance |                |            | Days to Bloom    |                |            | Flower diameter (cm) |                |            | No. of flowers per plant |                |            |
|--|------------------------|----------------|------------|------------------|----------------|------------|----------------------|----------------|------------|--------------------------|----------------|------------|
|  | V <sub>1</sub>         | V <sub>2</sub> | Mean       | V <sub>1</sub>   | V <sub>2</sub> | Mean       | V <sub>1</sub>       | V <sub>2</sub> | Mean       | V <sub>1</sub>           | V <sub>2</sub> | Mean       |
| T <sub>1</sub> (Control)                 | 53.25                  | 50.88          | 52.06      | 67.67            | 62.33          | 65.00      | 7.80                 | 7.37           | 7.58       | 90.00                    | 101.75         | 95.88      |
| T <sub>2</sub> (7 ppm nano FeS)          | 50.92                  | 50.92          | 50.92      | 65.67            | 62.33          | 64.00      | 7.82                 | 7.59           | 7.70       | 95.13                    | 108.17         | 101.65     |
| T <sub>3</sub> (14 ppm nano FeS)         | 51.17                  | 49.50          | 50.33      | 67.33            | 64.33          | 65.83      | 8.28                 | 7.57           | 7.92       | 116.88                   | 95.75          | 106.31     |
| T <sub>4</sub> (21 ppm nano FeS)         | 50.13                  | 50.50          | 50.31      | 68.00            | 65.00          | 66.50      | 7.53                 | 7.76           | 7.65       | 102.88                   | 115.00         | 108.94     |
| T <sub>5</sub> (28 ppm nano FeS)         | 49.63                  | 50.50          | 50.06      | 66.00            | 64.67          | 65.33      | 8.11                 | 7.56           | 7.83       | 106.02                   | 127.00         | 116.51     |
| T <sub>6</sub> (0.2% FeSO <sub>4</sub> ) | 49.33                  | 51.67          | 50.50      | 66.33            | 66.33          | 66.33      | 8.33                 | 7.85           | 8.09       | 102.88                   | 111.75         | 107.31     |
| T <sub>7</sub> (0.4% FeSO <sub>4</sub> ) | 50.50                  | 49.46          | 49.98      | 67.33            | 67.00          | 67.17      | 8.33                 | 7.14           | 7.74       | 91.63                    | 90.58          | 91.11      |
| T <sub>8</sub> (0.6% FeSO <sub>4</sub> ) | 51.08                  | 49.25          | 50.17      | 65.33            | 66.33          | 65.83      | 8.03                 | 7.23           | 7.63       | 115.00                   | 111.42         | 113.21     |
| T <sub>9</sub> (0.8% FeSO <sub>4</sub> ) | 48.50                  | 48.75          | 48.63      | 65.00            | 63.00          | 64.00      | 7.27                 | 7.60           | 7.43       | 131.92                   | 136.17         | 134.04     |
| <b>Mean</b>                              | <b>50.50</b>           | <b>50.16</b>   | -          | <b>66.52</b>     | <b>64.59</b>   | -          | <b>7.94</b>          | <b>7.52</b>    | -          | <b>105.81</b>            | <b>110.84</b>  | -          |
| <b>Factor</b>                            | <b>C.D. (5%)</b>       | <b>SEM</b>     | <b>SEM</b> | <b>C.D. (5%)</b> | <b>SEM</b>     | <b>SEM</b> | <b>C.D. (5%)</b>     | <b>SEM</b>     | <b>SEM</b> | <b>C.D. (5%)</b>         | <b>SEM</b>     | <b>SEM</b> |
| Variety (V)                              | NS                     | 0.538          | 0.655      | 1.891            | 0.655          | 0.032      | 0.093                | 0.032          | 0.032      | NS                       | 2.378          | 2.378      |
| Treatments (T)                           | 1.553                  | 0.254          | 1.390      | NS               | 1.390          | 0.068      | 0.197                | 0.068          | 0.068      | 14.559                   | 5.044          | 5.044      |
| V×T                                      | NS                     | 0.761          | 1.965      | NS               | 1.965          | 0.097      | 0.279                | 0.097          | 0.097      | NS                       | 7.133          | 7.133      |

**Table 3 : Effect of different concentrations of nano and macro forms of iron on flower characters in calendula varieties ‘Fiesta Gitana Mix (V<sub>1</sub>)’ and ‘Fiesta Yellow (V<sub>2</sub>)’**

| Treatments                               | Flower weight (g) |                |                  | Duration of flowering (days) |                  |            | Flower yield per plant (g) |                |                  | Flower yield (tons/ha) |                  |            |
|--|-------------------|----------------|------------------|------------------------------|------------------|------------|----------------------------|----------------|------------------|------------------------|------------------|------------|
|  | V <sub>1</sub>    | V <sub>2</sub> | Mean             | V <sub>1</sub>               | V <sub>2</sub>   | Mean       | V <sub>1</sub>             | V <sub>2</sub> | Mean             | V <sub>1</sub>         | V <sub>2</sub>   | Mean       |
| T <sub>1</sub> (Control)                 | 3.78              | 2.70           | 3.24             | 59.42                        | 65.00            | 62.21      | 328.69                     | 275.19         | 301.94           | 18.26                  | 15.29            | 16.77      |
| T <sub>2</sub> (7 ppm nano FeS)          | 4.10              | 3.16           | 3.63             | 61.42                        | 64.67            | 63.04      | 390.32                     | 395.25         | 392.78           | 21.68                  | 21.96            | 21.82      |
| T <sub>3</sub> (14 ppm nano FeS)         | 3.76              | 3.60           | 3.68             | 59.92                        | 62.83            | 61.38      | 485.23                     | 349.55         | 417.39           | 26.96                  | 19.42            | 23.19      |
| T <sub>4</sub> (21 ppm nano FeS)         | 4.12              | 2.37           | 3.25             | 58.92                        | 62.17            | 60.54      | 423.30                     | 272.95         | 348.13           | 23.52                  | 15.16            | 19.34      |
| T <sub>5</sub> (28 ppm nano FeS)         | 4.70              | 2.50           | 3.60             | 60.83                        | 62.33            | 61.58      | 498.45                     | 336.34         | 417.39           | 27.69                  | 18.69            | 23.19      |
| T <sub>6</sub> (0.2% FeSO <sub>4</sub> ) | 4.27              | 3.01           | 3.64             | 60.75                        | 60.75            | 60.75      | 486.54                     | 361.46         | 424.00           | 27.03                  | 20.08            | 23.56      |
| T <sub>7</sub> (0.4% FeSO <sub>4</sub> ) | 4.52              | 3.26           | 3.89             | 60.17                        | 60.00            | 60.08      | 455.07                     | 295.08         | 375.07           | 25.28                  | 16.39            | 20.84      |
| T <sub>8</sub> (0.6% FeSO <sub>4</sub> ) | 3.56              | 3.22           | 3.39             | 61.92                        | 60.75            | 61.33      | 422.25                     | 368.80         | 395.52           | 23.46                  | 20.49            | 21.97      |
| T <sub>9</sub> (0.8% FeSO <sub>4</sub> ) | 2.64              | 2.88           | 2.76             | 62.00                        | 64.08            | 63.04      | 362.40                     | 388.95         | 375.68           | 20.13                  | 21.61            | 20.87      |
| <b>Mean</b>                              | <b>3.94</b>       | <b>2.97</b>    | -                | <b>60.59</b>                 | <b>62.51</b>     | -          | <b>428.03</b>              | <b>338.17</b>  | -                | <b>23.78</b>           | <b>18.79</b>     | -          |
| <b>Factor</b>                            | <b>C.D. (5%)</b>  | <b>SEM</b>     | <b>C.D. (5%)</b> | <b>SEM</b>                   | <b>C.D. (5%)</b> | <b>SEM</b> | <b>C.D. (5%)</b>           | <b>SEM</b>     | <b>C.D. (5%)</b> | <b>SEM</b>             | <b>C.D. (5%)</b> | <b>SEM</b> |
| Variety (V)                              | 0.236             | 0.082          | 1.907            | 0.661                        | 24.607           | 8.525      | 1.367                      | 0.473          |                  |                        |                  |            |
| Treatments (T)                           | 0.5               | 0.173          | NS               | 1.402                        | 52.2             | 18.085     | 2.899                      | 1.004          |                  |                        |                  |            |
| V×T                                      | 0.707             | 0.245          | NS               | 1.982                        | 73.822           | 25.576     | 4.1                        | 1.42           |                  |                        |                  |            |

A perusal of data presented in Table 2 indicated the significant effect of treatments and non-significant effect of varieties as well as treatment-variety interaction on number of flowers. Irrespective of varieties, maximum number of flowers (134.04) was recorded in  $T_9$ , which was significantly higher than rest of the treatments, whereas, minimum number of flower (91.11) recorded in  $T_7$ . Enhancement in number of flowers might have attributed by increased leaf chlorophyll content, increased enzymatic activity in leaves, etc. as influenced by iron NPs in Durum wheat (Ghafari and Razmjoo, 2015) and in saffron plants (Farahani *et al.*, 2015).

The treatments, varieties and their interaction had significantly influenced the average flower weight, duration of flowering, flower yield per plant and flower yield (Table 3). Irrespective of varieties, average flower weight was recorded maximum (3.89 g) in  $T_7$  which was statistically at par with  $T_3$  (3.68 g),  $T_6$  (3.64 g),  $T_2$  (3.63 g)  $T_5$  (3.60 g) and  $T_8$  (3.39 g) but significantly higher than control ( $T_1$ ). However, it was recorded minimum (2.76 g) in  $T_9$ . The variety Fiesta Gitana Mix had significantly higher average flower weight (3.94 g) than variety Fiesta Yellow (2.97 g). Among interaction, maximum individual flower weight (4.70 g) was recorded in  $V_1T_5$  which was at par with  $V_1T_7$  (4.52 g),  $V_1T_6$  (4.27 g) and  $V_1T_2$  (4.10 g), whereas it was recorded minimum (2.37 g) in  $V_2T_4$ . Bakhtiari *et al.* (2015) reported enhanced spike weight of wheat due to application of nano iron oxide (0.04%). Higher concentration of NPs (1000ppm) enhanced plant growth in *Hydrangea paniculata* (Karunakaran *et al.*, 2017).

Non-significant effect of treatments, treatment-variety interaction but significant effect of varieties on duration of flowering was observed. The variety Fiesta Yellow had significantly higher duration of flowering (62.51 days) than variety Fiesta Gitana Mix (60.59 days).

Irrespective of varieties, maximum flower yield per plant (424g) was recorded in  $T_6$  which was at par with all the treatments but significantly higher than control whereas minimum flower yield per plant (301.94 g) was recorded in control ( $T_1$ ). Barring treatments, variety Fiesta Gitana Mix ( $V_1$ ) had significantly higher flower yield per plant (428.03 g) over variety Fiesta Yellow ( $V_2$ ) (338.17 g). Maximum flower yield per plant among interactions (498.45 g) was recorded in

$V_1T_5$  which was statistically at par with  $V_1T_6$  (486.54 g)  $V_1T_3$  (485.23 g)  $V_1T_7$  (455.07 g) but significantly higher than remaining treatments and control in both the varieties. However, the minimum flower yield per plant (272.95 g) was recorded in  $V_2T_4$ . These significant results might be due to reduced nutrient loss and strong adsorption ability as reported by Hu *et al.* (2017) and increased ability of plants to overcome stressed conditions (Elfeky *et al.*, 2013).

Irrespective of varieties, estimated flower yield was recorded highest in  $T_6$  (23.56 t/ha) which was at par with all the treatments except control ( $T_1$ ) and  $T_4$  (19.34 t/ha) with control ( $T_1$ ) being recorded for least flower yield (16.77 t/ha). The variety Fiesta Gitana Mix had significantly higher estimated flower yield (23.78 t/ha) than variety Fiesta Yellow (18.79 t/ha). Among interaction estimated flower yield was recorded maximum (27.69 t/ha) in  $V_1T_5$  which was statistically at par with  $V_1T_6$  (27.03 t/ha),  $V_1T_3$  (26.96 t/ha) and  $V_1T_7$  (25.28 t/ha) but significantly higher than rest of the treatments. However, it was found minimum (15.16 t/ha) in  $V_2T_4$ . This might be due to increased nutrients uptake and enhanced enzymatic activities in peppermint (Askary *et al.*, 2017) and increase in biomass production with iron application (Torabian *et al.*, 2018), where, foliar application of  $FeSO_4$  in nano and normal form increased leaf area, shoot dry weight, net carbon dioxide ( $CO_2$ ) assimilation rate, substomatal  $CO_2$  concentration, chlorophyll content, iron (Fe) content and decreased sodium (Na) content in leaves of sunflower.

The data on test weight of seeds showed significant effect of treatments, varieties and their interactions (Table 4). Irrespective of varieties, test weight of seeds was recorded highest in  $T_9$  (15.63 g) which was statistically at par with treatments  $T_6$  (15.25 g),  $T_7$  (15.22 g) and  $T_4$  (14.90 g) but significantly higher than rest of the treatments. However, it recorded minimum in control ( $T_1$ ) (12.84 g). Barring treatments, variety Fiesta Gitana Mix ( $V_1$ ) had significantly higher test weight of seeds (14.65 g) than variety Fiesta Yellow ( $V_2$ ) (14.11 g). Among interaction, maximum test weight of seeds (16.10 g) was recorded in  $V_1T_8$ , which was at par with  $V_1T_9$  (16.10 g),  $V_1T_6$  (15.72 g),  $V_2T_7$  (15.69 g),  $V_2T_5$  (15.67 g) but significantly higher than rest of the treatments combinations and was minimum (12.64) in control. Increased test weight of seeds was observed with increasing concentration of nano iron particles. It may be opined that enhanced test weight

**Table 4 : Effect of different concentrations of nano and macro forms of iron on seed yield and oil content in calendula varieties ‘Fiesta Gitana Mix (V<sub>1</sub>)’ and ‘Fiesta Yellow (V<sub>2</sub>)’**

| Treatments                               | Test weight of seeds (g) |                |       | Oil content in seeds (%) |                |            |
|--|--------------------------|----------------|-------|--------------------------|----------------|------------|
|  | V <sub>1</sub>           | V <sub>2</sub> | Mean  | V <sub>1</sub>           | V <sub>2</sub> | Mean       |
| T <sub>1</sub> (Control)                 | 13.04                    | 12.64          | 12.84 | 11.75                    | 12.84          | 12.30      |
| T <sub>2</sub> (7 ppm nano FeS)          | 13.20                    | 12.82          | 13.01 | 12.67                    | 13.74          | 13.20      |
| T <sub>3</sub> (14 ppm nano FeS)         | 13.45                    | 13.93          | 13.69 | 12.37                    | 14.34          | 13.35      |
| T <sub>4</sub> (21 ppm nano FeS)         | 15.40                    | 13.15          | 14.27 | 12.43                    | 15.44          | 13.93      |
| T <sub>5</sub> (28 ppm nanoFeS)          | 14.14                    | 15.67          | 14.90 | 14.69                    | 15.03          | 14.86      |
| T <sub>6</sub> (0.2% FeSO <sub>4</sub> ) | 15.72                    | 14.78          | 15.25 | 12.03                    | 12.85          | 12.44      |
| T <sub>7</sub> (0.4% FeSO <sub>4</sub> ) | 14.75                    | 15.69          | 15.22 | 13.49                    | 14.28          | 13.88      |
| T <sub>8</sub> (0.6% FeSO <sub>4</sub> ) | 16.10                    | 13.12          | 14.61 | 12.39                    | 13.91          | 13.15      |
| T <sub>9</sub> (0.8% FeSO <sub>4</sub> ) | 16.07                    | 15.20          | 15.63 | 13.53                    | 13.27          | 13.40      |
| <b>Mean</b>                              | 14.65                    | 14.11          | -     | 12.81                    | 13.97          | -          |
| <b>Factor</b>                            | <b>C.D. (5%)</b>         | <b>SEm</b>     |       | <b>C.D. (5%)</b>         |                | <b>SEm</b> |
| Variety (V)                              | 0.322                    | 0.111          |       | 0.496                    |                | 0.174      |
| Treatments (T)                           | 0.682                    | 0.236          |       | 1.051                    |                | 0.369      |
| V×T                                      | 0.965                    | 0.334          |       | NS                       |                | 0.522      |



of seeds may be attributed by more accumulation of iron in seeds (Rawat, 2017). Ghafari and Razmjoo (2015) reported increased 1,000 grain-weight due to application of nano iron in wheat.

The oil content of seeds was significantly affected by variety and treatment but non-significantly affected by variety-treatment interaction. Irrespective of varieties, oil content of seeds was recorded highest in T<sub>5</sub> (14.86%) and it was at par with T<sub>4</sub> (13.93%) and T<sub>7</sub> (13.88%) but significantly higher than rest of the treatments. However, minimum oil content (12.30%) was obtained in control (T<sub>1</sub>). The variety Fiesta Yellow (V<sub>2</sub>) had significantly higher oil content of seeds (13.97%) than variety Fiesta Gitana Mix (V<sub>1</sub>) (12.81%). It is apparent that higher seed oil content of calendula was obtained from nano iron treatments with lesser concentrations. The treatment of 21 ppm nano FeS with variety Fiesta Yellow had performed better for seed oil content and was significantly higher than result obtained with normal or macro iron among both varieties. Also, variety Fiesta Yellow had recorded significant result though all other flowering attributes were lesser than variety Fiesta Gitana Mix (V<sub>1</sub>) and can be considered with perspective of seed oil of calendula. The increased oil content due to nano iron application in calendula (Amuamuha *et al.*, 2012) and in chamomile (Elfeky *et al.*, 2013) was observed.

## REFERENCES

- Alamdari, S.E., Rezaei, M.A., Farahvash, F. and Janlou, M.M. 2021. Effects of nano-potassium and potassium sulfate fertilizers and salicylic acid on the morpho-physiological traits of marigold, *Calendula officinalis* under drought stress. *International Journal of Modern Agriculture*, **10**(1): 286-297.
- Amuamuha, L., Pirzad, A. and Hadi, H. 2012. Effect of varying concentrations and time of nano iron foliar application on the yield and essential oil of pot marigold. *International Research Journal of Applied and Basic Sciences*, **3** (10): 2085-2090.
- Askary, M., Amirjani, M. R., and Saberi, T. 2017. Comparison of the effects of nano-iron fertilizer with iron-chelate on growth parameters and some biochemical properties of *Catharanthus roseus*. *Journal of Plant Nutrition*, **40**(7): 974-982
- Bakhtiari, M., Moaveni, P. and Sani, B. 2015. The effect of iron nanoparticles spraying time and concentration on wheat. *Biological Forum – An International Journal*, **7**(1): 679-683.
- Elfeky, S. A., Mohammed, M. A., Khater, M. S., Osman, Y. A. H. and Elsherbini, E. 2013. Effect of magnetite nano-fertilizer on growth and yield of *Ocimum basilicum* L. *International Journal of Indigenous Medicinal Plants*, **46**(3):2051-4263.
- Farahani, S. M., Khalesi, A. and Sharghi, Y. 2015. Effect of nano iron chelate fertilizer on iron absorption and saffron (*Crocus sativus* L.) quantitative and qualitative characteristics. *Asian Journal of Biological Sciences*, **8** (2): 72-82.
- Ghafari, H. and Razmjoo, J. 2015. Response of durum wheat to foliar application of varied sources and rates of iron fertilizers. *Journal of Agriculture Science and Technology*, **17**:321-331.
- Goshwami, V., Srivastava, R., Bhandari, N.S., Kumar, A., Tewari, S. and Gangola, S. 2021. Dynamic interventions of growth regulation in calendula (*Calendula officinalis* L.) as influenced by gold-nanoparticle. *Plant Archive*. **21**(1): 2591-2595.
- Hu, J., Guo, H., Li, J., Wang, Y., Xiao, L. and Xing, B. 2017. Interaction of  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub> nanoparticles with *Citrus maxima* leaves and the corresponding physiological effects via foliar application. *Journal of Nanobiotechnology*, **15**:51.
- Karunakaran, G., Jagathambal, M., Minh, N. V., Kolesnikov, E., Gusev, A., Zakharova, O. V., Scripnikova, E. V., Vishnyakova, E. D. and Kuznetsov, D. 2017. Green synthesized iron oxide nanoparticles: a nano-nutrient for the growth and enhancement of flax (*Linum usitatissimum* L.) plant. *International Journal of Biotechnology and Bioengineering*, **11**(4): 289-293.
- Khalid, K. A. and Teixeira da Silva, J. A. 2010. Yield, essential oil and pigment content of *Calendula officinalis* L. flower heads cultivated under salt stress conditions. *Scientia Horticulturae*, **126**:297–305.
- Król, B. and Paszko, T. 2017. Harvest date as a factor affecting crop yield, oil content and

- fatty acid composition of the seeds of calendula (*Calendula officinalis* L.) cultivars. *Industrial Crops and Products*, **97**:242–251.
- Mohammadi, H., Hatami, M., Feghezadeh, K. and Ghorbanpour, M. 2018. Mitigating effect of nano-zerovalent iron, iron sulfate and EDTA against oxidative stress induced by chromium in *Helianthus annuus* L. *Acta Physiologiae Plantarum*, **40**:69
- Paolini, J., Barboni, T., Desjobert, J., Djabou, N., Muselli, A. and Costa, J. 2010. Chemical composition, intraspecies variation and seasonal variation in essential oils of *Calendula arvensis* L. *Biochemical Systematics and Ecology*, **38**:865–874.
- Pirzad, A. and Shokrani, F. 2012. Effects of iron application on growth characters and flower yield of *Calendula officinalis* L. under water stress. *World Applied Sciences Journal*, **18** (9): 1203-1208.
- Praveen, A., Khan, E., Ngiime S. D., Perwez, M., Sardar, M. and Gupta, M. 2018. Iron oxide nanoparticles as nano-adsorbents: a possible way to reduce arsenic phytotoxicity in Indian mustard plant (*Brassica juncea* L.). *Journal of Plant Growth Regulation*, **37**:612–624.
- Rawat, M., Nayan, R., Negi, B., Zaidi, M.G.H. and Arora, S., 2017. Physio-biochemical basis of iron-sulfide nanoparticle induced growth and seed yield enhancement in *B. juncea*. *Plant Physiology and Biochemistry*, **118**:274-284.
- Saffari, V.R. and Saffari M. 2020. Effects of EDTA, citric acid, and tartaric acid application on growth, phytoremediation potential, and antioxidant response of *Calendula officinalis* L. in a cadmium-spiked calcareous soil. *International Journal of Phytoremediation*. 1204-1214. (<https://doi.org/10.1080/15226514.2020.1754758>).
- Sahingil, D. 2019. GC/MS-Olfactometric Characterization of the volatile compounds, determination antimicrobial and antioxidant activity of essential oil from flowers of calendula (*Calendula officinalis* L.). *Journal of Essential Oil-Bearing Plants*. **22**(6): 1571-1580.
- Salarpour, O., Parsa, S., Sayyari, M. H., Alahmadi, M. J. 2013. Effect of nano-iron chelates on growth, peroxidase enzyme activity and oil essence of cress (*Lepidium sativum* L.). *International Journal of Agronomy and Plant Production*, **4**: 3583-3589.
- Tayade, M., Badge, S. and Nikam, B. 2018. Foliar application of zinc and iron as influenced on flowering and quality parameters of tuberose. *International Journal of Current Microbiology and Applied Sciences*, **7**(1): 2239-2243.
- Torabian, S., Farhangi-Abriz, S. and Zahedi, M. 2018. Efficacy of FeSO<sub>4</sub> nano formulations on osmolytes and antioxidative enzymes of sunflower under salt stress. *Indian Journal of Plant Physiology*, **23**(2):305–315.
- Yuan, J., Chen, Y., Li, H., Lu, J., Zhao, H., Liu, M., Nechitaylo, G. S. and Glushchenko, N. N. 2018. New insights into the cellular responses to iron nanoparticles in *Capsicum annuum*. *Scientific Reports*, **8**:3228.