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

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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 % FeSO₄ 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 FeSO₄ 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'.

Keywords: Calendula, Ferrous sulphate, Flowering, Nano iron, Oil content, 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* región 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-1, respectively. Well rotten farmyard manure @ 5 kg/m² 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

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 FeSO₄ 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 FeSO₄ recorded significantly more number of branches (26.75) in T₉ than rest of the treatments, however, it was recorded minimum (14.50) in control (T₁). 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 V₁T₉ followed by V₂T₆ (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 (V₁T₂). Torabian *et al.* (2018) reported that increased growth in sunflower grown under saline condition by application of FeSO₄ both in normal and nano form which is due to increased leaf area, net CO₂ assimilation, sub-stomatal CO₂ 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. However, number of branches was recorded minimum (12.00) in variety Fiesta Gitana Mix sprayed with 21 ppm nano FeS (V₁T₂). Torabian *et al.* (2018) reported that increased growth in sunflower grown under saline condition by application of FeSO₄ both in normal and nano form which is due to increased leaf area, net CO₂ assimilation, sub-stomatal CO₂

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 lants 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 T₉ (29.73 cm) which was at par with T₈ (29.42 cm) and T₇ (27.86 cm) but significantly higher than the rest of the treatments. However, minimum was recorded in T₂ (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 V₁T₉ combination followed by V₁T₈ (30.84), and V₁T₇ (30.34) and it was minimum (20.54 cm) in V₂T₂. Treatmnts T₉ (31.96 cm) and T₈ (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, T₅ (24.75cm) and T₄ (22.17 cm) had maximum plant height in variety Fiesta Gitana Mix (V₁) and Fiesta Yellow (V₂), 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 V₁’ and ‘Fiesta Yellow V₂’

Treatments	No. of branches			Plant height (cm)			Plant spread (cm)			Number of leaves		
	V1	V2	Mean	V1	V2	Mean	V1	V2	Mean	V1	V2	Mean
T1 (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
T2 (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
T3 (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
T4 (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
T5 (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
T6 (0.2% FeSO ₄)	21.92	25.17	23.54	27.46	25.71	26.59	41.75	44.67	43.21	26.92	17.33	22.13
T7 (0.4% FeSO ₄)	23.83	24.83	24.33	30.34	25.37	27.86	43.13	42.83	42.98	23.75	19.58	21.67
T8 (0.6% FeSO ₄)	24.75	23.17	23.96	30.84	28.00	29.42	44.75	43.79	44.27	26.83	19.00	22.92
T9 (0.8% FeSO ₄)	29.58	23.92	26.75	31.96	27.50	29.73	45.38	44.96	45.17	25.42	19.83	22.63
Mean	18.45	20.07		26.18	23.63		41.88	41.71		22.21	18.48	
Factor	C.D. (5%)		SEm	C.D. (5%)		SEm	C.D. (5%)		SEm	C.D. (5%)		SEm
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 FeO₃ (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 T₉ which was statistically at par with T₈ (44.27cm), T₆ (43.21cm) and T₇ (42.98cm) but significantly higher than rest of the treatments. However, minimum plant spread (39.19cm) was recorded in both, T₃ and T₅. 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 V₁T₉ followed by V₂T₉ (44.96 cm), V₂T₈ (44.75cm) and V₂T₆ (44.67cm). However, least plant spread (37.00 cm) was recorded in V₂T₅. Among nanoparticles treatments, T₅ in variety Fiesta Gitana Mix and T₂ 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, granastacking 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 T₈ which was statistically at par with T₆ (22.13) T₇ (21.67) and T₉ (22.63) but significantly higher than rest of the treatments. However, minimum number of leaves (18.04) was recorded in T₃. 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 V₁T₆ combination followed by V₁T₈ (26.83) and V₁T₉ (25.42) whereas least number of leaves (17.25) was recorded in V₂T₅. However, higher dose of nanoparticle resulted in more number of leaves. Praveen et al. (2018) reported improved growth of mustard plants treated with NPs (Fe₃O₄) 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 (T₁) which was statistically at par with by T. (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 T₉. Tayade et al. (2018) reported early initiation of spike in tuberose with 0.4% FeSO₄.

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% FeSO₄. 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 the varieties, maximum number of leaves (22.92) was recorded in T₈ which was statistically at par with T₆ (22.13) T₇ (21.67) and T₉ (22.63) but significantly higher than rest of the treatments. However, minimum number of leaves (18.04) was recorded in T₆. 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 V₁T₉ first floret in tuberose with 0.4% FeSO₄. 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 T₆ which statistically par with T₁ (7.92 cm) but significantly higher than rest of the treatments, whereas, minimum diameter of flower (7.43 cm) was recorded in T₁. 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 V₁T₆ and V₁T₇ and were statistically at par with V₁T₃ (8.28 cm) V₁T₅ (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 FeSO₄ (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 V₁' and 'Fiesta Yellow V₂'

Treatments	Days to bud appearance			Days to Bloom			Flower diameter (cm)			No. of flowers per plant		
	V1	V2	Mean	V1	V2	Mean	V1	V2	Mean	V1	V2	Mean
T1 (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
T2 (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
T3 (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
T4 (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
T5 (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
T6 (0.2% FeSO ₄)	49.33	51.67	50.50	66.33	66.33	66.33	8.33	7.85	8.09	102.88	111.75	107.31
T7 (0.4% FeSO ₄)	50.50	49.46	49.98	67.33	67.00	67.17	8.33	7.14	7.74	91.63	90.58	91.11
T8 (0.6% FeSO ₄)	51.08	49.25	50.17	65.33	66.33	65.83	8.03	7.23	7.63	115.00	111.42	113.21
T9 (0.8% FeSO ₄)	48.50	48.75	48.63	65.00	63.00	64.00	7.27	7.60	7.43	131.92	136.17	134.04
Mean	50.50	50.16	-	66.52	64.59	-	7.94	7.52	-	105.81	110.84	-
Factor	C.D. (5%)		SEm	C.D. (5%)		SEm	C.D. (5%)		SEm	C.D. (5%)		SEm
Variety (V)	NS		0.538	1.891		0.655	0.093		0.032	NS		2.378
Treatments (T)	1.553		0.254	NS		1.390	0.197		0.068	14.559		5.044
V×T	NS		0.761	NS		1.965	0.279		0.097	NS		7.133

Table 3:
Effect of different concentrations of nano and macro forms of iron on flower characters in calendula varieties 'Fiesta Gitana Mix (V₁)' and 'Fiesta Yellow (V₂)'

Treatments	Flower weight (g)			Duration of flowering (days)			Flower yield per plant (g)			Flower yield (tons/ha)		
	V1	V2	Mean	V1	V2	Mean	V1	V2	Mean	V1	V2	Mean
T1 (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
T2 (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
T3 (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
T4 (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
T5 (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
T6 (0.2% FeSO ₄)	4.27	3.01	3.64	60.75	60.75	60.75	486.54	361.46	424.00	27.03	20.08	23.56
T7 (0.4% FeSO ₄)	4.52	3.26	3.89	60.17	60.00	60.08	455.07	295.08	375.07	25.28	16.39	20.84
T8 (0.6% FeSO ₄)	3.56	3.22	3.39	61.92	60.75	61.33	422.25	368.80	395.52	23.46	20.49	21.97
T9 (0.8% FeSO ₄)	2.64	2.88	2.76	62.00	64.08	63.04	362.40	388.95	375.68	20.13	21.61	20.87
Mean	3.94	2.97	-	60.59	62.51	-	428.03	338.17	-	23.78	18.79	-
Factor	C.D. (5%)		SEm	C.D. (5%)		SEm	C.D. (5%)		SEm	C.D. (5%)		SEm
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 T₉ which was significantly higher than rest of the treatments, whereas, minimum

number of flower (91.11) recorded in T₇. 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 (3.89g) in T₇ which was statistically at par with T₃ (3.68g), T₆ (3.64 g), T₂ (3.63 g) T₅ (3.60 g) and T₈ (3.39g) but significantly higher than control (T₁). However, it was recorded minimum (2.76 g) in T₉. 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 V₁T₅ which was at par with V₁T₇ (4.52 g), V₁T₆ (4.27 g) and V₁T₂ (4.10 g), whereas it was recorded minimum (2.37 g) in V₂T₄. 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 T₆ 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 (T₁). Barring treatments, variety Fiesta Gitana Mix (V₁) had significantly higher flower yield per plant (428.03 g) over variety Fiesta Yellow (V₂) (338.17 g). Maximum flower yield per plant among interactions (498.45 g) was recorded V₁T₅ which was statistically at par with V₁T₆ (486.54 g) V₁T₃ (485.23 g) V₁T₇ (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 V₂T₄. 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 T₆ (23.56 t/ha) which was at par with all the treatments except control (T₁) and T₄ (19.34 t/ha) with control (T₁) 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 V₁T₅ which was statistically at par with V₁T₆ (27.03 t/ha), V₁T₃ (26.96 t/ha) and V₁T₇ (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_1 ' and 'Fiesta Yellow V_2 '

Treatments	Test weight of seeds (g)			Oil content in seeds (%)		
	V1	V2	Mean	V1	V2	Mean
T1 (Control)	13.04	12.64	12.84	11.75	12.84	12.30
T2 (7 ppm nano FeS)	13.20	12.82	13.01	12.67	13.74	13.20
T3 (14 ppm nano FeS)	13.45	13.93	13.69	12.37	14.34	13.35
T4 (21 ppm nano FeS)	15.40	13.15	14.27	12.43	15.44	13.93
T5 (28 ppm nano FeS)	14.14	15.67	14.90	14.69	15.03	14.86
T6 (0.2% $FeSO_4$)	15.72	14.78	15.25	12.03	12.85	12.44
T7 (0.4% $FeSO_4$)	14.75	15.69	15.22	13.49	14.28	13.88
T8 (0.6% $FeSO_4$)	16.10	13.12	14.61	12.39	13.91	13.15
T9 (0.8% $FeSO_4$)	16.07	15.20	15.63	13.53	13.27	13.40
Mean	14.65	14.11	-	12.81	13.97	-
Factor	C.D. (5%)		SEm	C.D. (5%)		SEm
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_5 (14.86%) and it was at par with T_4 (13.93%) and T_7 (13.88%) but

significantly higher than rest of the treatments. However, minimum oil content (12.30%) was obtained in control (T_1). The variety Fiesta Yellow (V_2) had significantly higher oil content of sedes (13.97%) than variety Fiesta Gitana Mix (V_1) (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_1) 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.

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