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DYNAMIC INTERVENTIONS OF GROWTH REGULATION IN CALENDULA (*CALENDULA OFFICINALIS* L.) AS INFLUENCED BY GOLD-NANOPARTICLE

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ABSTRACT

Nanotechnology is the art and science of manipulating material at atomic scale. It can also be defined as the designing, characterization, production and application of structures, devices and systems by controlling shape and size at the nanoscale. The present investigation was carried out to assess the effect of Gold-nanoparticle treatment on vegetative and flowering attributes of Calendula. Experiment consisted of four treatments (5, 10, 15, 20 ppm Gold-nanoparticle), along with control and was laid out in Randomized block design. Among the various treatments, application of 10 ppm of Gold-nanoparticle (T_2) was found best for most of the parameters *viz.,* plant height (73.95 cm), plant spread (54.62 cm), number of branches (25.15), number of leaves (127.55), number of flower (131.30), flower diameter (7.06 cm), flower weight (4.31), minimum days to flower bud initiation (30.85 days) and Flowering duration (105.15 days over the rest treatments. Treatment T_4 (20 ppm Gold-nanoparticle) showed poor growth in most of the recorded parameters due to the toxicity of Gold-nanoparticle at higher concentration. Nanoparticles when applied to the plants can help them to cope up the adverse condition by releasing the ROS enzyme (Reverse Oxygen Species). During stress conditions ROS enzyme releases and antioxidant defense system of plant gets activated to maintain the normal equilibrium. As a result additional amount of proteins, carbohydrates and DNAs are formed. These additional molecules enhance plant growth and improve yield.

***Keywords*:** Nanotechnology, Gold-nanoparticle, Calendula, Vegetative and Reproductive attributes.

Introduction

Calendula (*Calendula officinalis)* commonly known as calendula, pot marigold, Mary's gold, or poor man's saffron belongs to the family Asteraceae. Because the flowers follow the sun, it was linked to the astrological sign of summer. It is an annual herb with yellow to orange flowers, native to Mediterranean region (Gazim *et al*., 2008). Commercially, calendula is extensively grown in beds, baskets and boxes. Flowers are also gaining popularity as cut flower, and traditionally used for offering in churches and temples for use in ceremonies, festivals, beautification and landscape plans. Besides this, calendula is known for its various medicinal properties like anti-inflammatory, antiseptic, anti-viral and anti-bacterial properties and helps in boosting the immune system. The flower contains essential oil which is used for high blood-fat and treatment of inflammation of intestinal organs (Mrda *et al*., 2007). Calendula has been for long a popular domestic remedy for skin problems ranging from bites and stings to wounds healing. It is also helpful in regulating menstrual cycle & reduces fever. It stimulates white blood cells which destroys harmful microbes. It is a cleaning and detoxifying herb and is taken externally for getting relief from several medical conditions like stomach ulcer, menstrual cramps, liver disease, tooth-ache, eye inflammation, persistent constipation and also worm infestation. This herb also helps in controlling insects when grown in vegetable garden. Flower buds and leaves are extensively used in homoeopathic medicines also. Besides medicinal uses, it is also used in perfume-making and its yellow colour pigment carotenoids is used in cosmetics as a colouring element.

With the fast emerging population, arable land is diminishing day by day. In this scenario, increasing crop yield on the limited area is becoming a prime goal. Scientists are emphasizing more on the proper utilization of limited resources available. For which, new technologies like genetically modified (GM) crops and nanotechnology can be applied to improve crop yield. Nanoparticles because of their no or very less environmental hazard can be used as a beneficial alternative in enhancing growth and productivity. Nanotechnology, a new emerging and fascinating field of science, permits advance research in many areas. Nano-technological discoveries could open up novel application in the field of biotechnology and agriculture. Nanoparticles have unique physico-chemical properties and the potential to boost the plant metabolism (Giraldo *et al.,* 2014). Nanotechnology can also stimulate plant growth. Nanoparticles when applied to the plants can help them to cope up the adverse conditions by releasing the ROS enzyme (Reactive Oxygen Species). Nanofertilzers or nanoencapsulated nutrients might have properties that are effective to crops, released the nutrients on-demand, controlled release of chemicals, fertilizers that regulate plant growth and enhanced target activity (DeRosa *et al*., 2010 and Nair *et al.*, 2010). A variety of engineered nanoparticles are being used in past few decade to improve crop yield. (Scrinis and Lyons 2007; Barik *et al.,* 2008). Some studies suggest that AuNPs induces toxicity in plants by inhibiting aquaporin (a group of proteins that help in the transportation of wide range of molecules including water) function (Shah and Belozerova, 2009). However, contrasting results have been observed by other researchers. Gold nanoparticles are found to increase plant height, number of leaves per plant, leaf area, chlorophyll content, and sugar content resulting in the better crop yield (Arora *et al*., 2012). Few reports suggest that AuNPs play significant role in the germination of micro RNA expression which regulates various morphological, physiological and metabolic processes in plants. (Kumar *et al.,* 2013).The positive effects of gold nanoparticles on the plant need further study. In this respect, the present investigation planned with the objectives to assess the effect of various concentrations of gold nanoparticles on vegetative and flowering attributes of calendula.

Material and Methods

The present investigation entitled "Dynamic interventions of growth regulation in Calendula (*Calendula officinalis* L.) as influenced by gold-nanoparticle" was carried out at Model Floriculture Centre, G.B. Pant University of Agriculture and Technology, Pantnagar during winter season. Pantnagar is situated in the *Tarai* region of Uttarakhand at 29^0 N latitude and 79.3^0 E longitudes in the foot hills of the Himalaya at an altitude of 243.84 m above mean sea level. The experimental material for the present investigation comprises of calendula single type and double type. During vegetative growth, plants were treated with nano-gold suspension of different concentrations (5, 10, 15 and 20 ppm). The experiment was laid out in randomized block design (RBD) with four replications and five treatments. All the plants used were uniform in growth. Five plants per treatment per replication were randomly selected for observations. Observations were taken on randomly selected five plants of each treatment to assess the effect of nanogold treatments on plant height, plant spread, number of branches per plant, number of leaves per plant, days to flowering, duration of flowering, diameter of flower, total number of flowers per plant and weight of flower at full bloom.Data recorded was statistically analysed with the help of computer by using SAS software application. The significance of variance among the treatment was observed by applying 'F' test and "T" tests and critical difference at 5% level of probability. The table for analysis of variance (ANOVA) was set as explained by Gomez and Gomez (1984).

The following formula was used to calculate CD.

$$CD = S.\ E._{diff.}\ X\ t\ (5\%\ at\ error\ degree\ of\ freedom)$$

Where:

- t = Tabulated value of 't' at 5 per cent level of significance at error degree of freedom
- S. $E._{diff.}$ = standard error of difference of the two treatments means to be sampled which is calculated as following:

$$S.\ E._{diff.} = \sqrt{\frac{2MSE}{r}}$$

Where:

- MSE = Mean square of error and
- R = Number of replications.

Result and Discussion

Nanotechnological discoveries could open up novel application in the field of biotechnology and agriculture. Nanoparticles have unique physico-chemical properties and the potential to boost the plant metabolism (Giraldo *et al.* 2014). During the investigation, data were recorded on various aspects of growth and flowering. The findings of the work have been tabulated and discussed for the effect of various treatments of Gold-nanoparticle on the plant with regards to different attributes studied by other scientists. The results emanating from the current investigation are presented under following heads: Significantly higher plant height, plant spread, number of branches per plant, number of leaves per plant, diameter of flower, total number of flowers per plant and weight of flower at full bloom, were recorded with the application of 10 ppm Gold-nanoparticle (T_2) which proved their superiority over the rest treatments for growth attributes enhancement at different growth stages.

The data pertaining to the effect of different concentrations (T_1: 5 ppm, T_2: 10 ppm, T_3: 15 ppm andT_4:20 ppm) of Gold nanoparticle over control treatment (T_5: distilled water) on plant height of calendula are presented in Table (1). After 100 days of application of Gold-nanoparticle the maximum plant height (73.95 cm) was recorded with T_2 that was significantly higher than rest of the treatments and followed by T_1 (64.95 cm),T_3 (61.75 cm), T_5 (61.20 cm), however; the shortest plant height was recorded in treatment T_4 (57.20 cm). Findings of present investigation suggested that, maximum plant height was recorded with lowest concentration of Gold-nanoparticle (AuNP) treatment (10 ppm), this might be due to increased chlorophyll content (Arora *et al*., 2012) resulting in more photosynthetic activity which further facilitated the maximum production and utilization of photo-assimilates. Positive effects of nanoparticles are more pronounced at lower concentrations. The increment in the height might be due to increased level of Gibberellic acid (GAs), as GA is responsible for shoot elongation (Stepanova *et al.,* 2007). However, the lesser plant height was observed with the highest concentration (20 ppm) of AuNP, because at higher concentration AuNP become toxic to plants and inhibiting aquaporin function, a group of proteins that help in the transportation of wide range of molecules including water (Shah and Belozerova, 2009).

The similar trend of growth in plant spread was observed with highest value of spread in treatment T_2 (50.70 cm and 54.62 cm) that was statistically at par with T_5 (46.80 and 52.60 cm) and T_3 (47.15 and 51.25 cm) but significantly higher than T_1 (45.02 and 49.32 cm) and T_4 (40.17 and 43.75 cm) in respective growth stage of 80 and 100 days (Table 2). The interaction of plant cells with the engineered nanoparticles leads to modification in biological pathways and plant gene expression which eventually affects growth and development in plants (Ghormade *et al.,* 2011; Khiew *et al.,* 2011 and Hassan *et al.,* 2013). The increment in growth parameters might be due to the increased efficiency caused by application of nanoparticles.

The maximum number of branches were observed after 80 of spray with T_2 (18.75) which was statistically at par with other treatments T_1(17.35), T_3(18.65), T_4(18.45) and control T_5(18.45). The results of the investigation showed an increased number of branches at 10 ppm gold-nanoparticle treatment followed by control (Table 3). However; after 100 days of spray no significant increment in the number of branches was observed. For calendula crop 10 ppm concentration may be the dose which can increase the efficiency of the plant. Tripathi *et al.* (2011) recorded increased pattern of growth in number of branches by application of water soluble Carbon nanotubes (wsCNTs) at 6.0 µg/ml concentration in *Cicerarietinum.* Arora *et al.* (2012) also stated that application of Gold-nanoparticles increased the average number of secondary branches (36 %), while there was a reduction in primary branches upto 9% at 10 ppm to 25 % at 100 ppm.

It is evident from the data that number of leaves was positively influenced by treatment of Gold-nanoparticles. The maximum number of leaves was recorded in treatment T_2 (127.55) which was statistically at par with T_1 (112.55) and T_5 (107.20) but significantly higher than rest of the treatments (Table 4). However, minimum number of leaves was recorded in T_4 (89.50). In a plant, leaf number is regulated by a complex interaction of various genes whose expression is modulated by growth hormones (Gonzalez *et al*., 2010). Gaseous plant hormone ethylene regulates leaf abscission. It has been observed that inhibition of ethylene action reduces the event of abscission Seif *et al.* (2011). Also, it is reported that increase in number of leaves can be due to inhibition of ethylene action (Valdovinos *et al*., 1967). Arora*et al.* (2012) reported increase in number of leaves (about 25 %) by the application of gold nanoparticle treatment. Khodakovskaya *et al.* (2013) reported that treatment with 50 µg/ml carbon nanotubes (CNT) application in tomato plant increased number of leaves. The data presented in Table 4 reveals that application of gold-nanoparticle also enhanced the average number of flowers per treatment but the difference was non-significant among all the treatments and control. However, the maximum number of flowers were recorded in treatment T_2 (131.30) whereas minimum flowers were recorded in treatment T_4 (108.8). Khodakovskaya *et al.* (2013) also reported that application of carbon nanotubes (CNT) in tomato proved efficient for activation of reproductive system of plants lead to two times increased production of flowers and fruits (50 µg/ml).

Maximum flower diameter was observed in treatment T_2 (7.06 cm) which was statistically at par with T_3 (6.26 cm) and T_4 (5.83 cm) but significantly higher than rest of the treatments (Table 5). However; the minimum flower diameter was recorded in treatment T_1 (4.97 cm). Khodakovskaya *et al.* (2013) reported higher diameter of tomato fruit when treated with carbon nanotubes at 50 µg/ml. The maximum weight of flower was recorded in treatment T_2 (4.31 g) which was significantly higher than T_1 (3.64 g), T_5 (3.50 g) and T_3 (3.30 g) while the minimum weight (1.08 g) of flower is recorded in treatment T_4 (Table 5). However, the minimum flower weight (1.08 g) was recorded in T_4. With the increase in concentration (5 ppm and 10 ppm) weight of flower increased after which it started declining(15ppm and 20 ppm). The reason behind increase in weight might be due to more and more divergence of sugar and carbohydrates to the reproductive areas of the plant. These findings are in agreement with Laware and Raskar (2014) also reported increased fresh weight of onion seedlings (468.92 mg) after the application of zinc nanoparticles at 20 µg/ml. Almutairi *et al*. (2015) also reported increased fresh seedling weight of *Zea mays* (154 gm) after treatment with 2 mg/ml silver nanoparticle treatment over control after 12 days of treatment. Bakhtiari *et al.* (2015) also reported increase in weight of wheat spike at 0.04% concentration of nano-iron solution.

The days required for flower bud initiation was recorded maximum in treatment T_4 (34.85 days) which was statistically at par with T_1 (33.25 days), T_3 (32.80 days) and T_5 (33.25 days) while minimum days (30.85 days) to flower were recorded in T_2 (Table 6). Results clearly stated that Gold- nanoparticle positively influenced the emergence of flower buds in calendula. Stepanova *et al.* (2007) reported that indigenous plant hormone auxin and ethylene can reciprocally regulate each other's response pathways, and act on the same target but independent of each other Application of Gold-nanoparticle had shown significant increase in number of branches which might be due to ethylene inhibition which can reciprocally induce higher auxin translocation in plant. Early flowering might be as a result of increased level of auxin and rapid translocation of sugars to the reproductive areas of the plant (Cheng and Zhao, 2007; Sundberg and Ostergaard, 2009). These results were in agreement with that of Raskar and Laware (2014) who also reported early induction of flowers by application of Zinc oxide nanoparticle (ZnONPs) at 20 and 30 µg/ml in onion (*Allium cepa*).

The data presented in Table 6 envisages that duration of flowering was significantly influenced by the application of Gold nanoparticle treatment. The maximum duration (105.15 days) of flowering was recorded in treatment T_2 which was significantly higher than rest of the treatments. The minimum duration (95.64 days) of flowering was recorded in treatment T_1. Like other flowering characters, a long duration of flowering was observed in 10 ppm Gold-nanoparticle treatment. The increased flowering duration might be due to the increased efficiency of plant caused by application of Gold nanoparticle. Das *et al*., 2018 suggested that efficiency increment may be a reason behind accelerated photosynthesis, resulting in more and more divergence of photo-assimilates to reproductive organs in all stages of plant growth resulting in longer duration of flowering. Thesimilar types of findings were also reported by Chavan *et al*., 2019.

Table 1: Effect of Gold-nanoparticles treatment on plant height of Calendula

Treatments	Plant Height (cm)				
	20 days	40 days	60 days	80 days	100 days
T_1(5 ppm)	31.55	44.35	53.55	60.90	64.95
T_2(10 ppm)	32.90	47.75	59.00	69.10	73.95
T_3(15 ppm)	28.80	39.50	47.80	57.40	61.75
T_4(20 ppm)	25.60	35.50	43.90	52.75	57.20
T_5(control)	31.35	41.25	49.25	56.60	61.20
CD 5%	4.19	5.82	7.10	7.01	7.17
SEm	1.36	1.88	2.30	2.27	2.32

Table 2: Effect of Gold-nanoparticle treatment on plant spread of Calendula

Treatments	Plant spread (cm)				
	20 days	40 days	60 days	80 days	100 days
T_1(5 ppm)	30.45	35.85	40.80	45.02	49.32
T_2(10 ppm)	32.35	39.35	45.57	50.70	54.62
T_3(15 ppm)	30.22	34.80	41.65	47.15	51.25
T_4(20 ppm)	24.42	29.75	35.35	40.17	43.75
T_5(control)	30.77	35.92	45.52	46.80	52.60
CD 5%	3.29	4.42	4.88	4.63	4.40
SEm	1.07	1.43	1.58	1.50	1.43

Table 3: Effect of Gold-nanoparticle treatment on number of branches of Calendula

Treatments	Plant spread (cm)				
	20 days	40 days	60 days	80 days	100 days
T_1(5 ppm)	3.00	6.85	12.75	17.35	22.80
T_2(10 ppm)	3.70	7.70	13.90	18.75	25.15
T_3(15 ppm)	3.20	7.85	13.88	18.65	24.00
T_4(20 ppm)	3.00	7.15	13.35	18.10	23.60
T_5(control)	3.20	7.30	14.15	18.45	24.55
CD 5%	0.77	0.97	1.42	3.22	3.34
SEm	0.25	0.31	0.46	1.04	1.08

Table 4: Effect of Gold-nanoparticle treatment on number of leaves and number of flowers of Calendula

Treatments	Number of leaves	Number of Flower
T_1(5 ppm)	112.55	127.65
T_2(10 ppm)	127.55	131.30
T_3(15 ppm)	94.05	122.10
T_4(20 ppm)	89.50	108.85
T_5(control)	107.20	121.35
CD 5%	24.51	32.16
SEm	7.95	10.43

Table 5: Effect of Gold-nanoparticle treatment of flower diameter and flower weight of Calendula

Treatments	Flowering attributes	
	Flower diameter (cm)	Flower weight (g)
T_1(5 ppm)	4.97	3.64
T_2(10 ppm)	7.06	4.31
T_3(15 ppm)	6.26	3.30
T_4(20 ppm)	5.83	1.08
T_5(control)	5.32	3.50
CD 5%	1.27	0.60
SEm	0.41	0.19

Table 6: Effect of Gold-nanoparticle treatment on days to flowering and duration of flowering of Calendula

Treatments	Flowering attributes	
	Days to flowering (days)	Flowering duration (days)
T_1(5 ppm)	33.25	95.64
T_2(10 ppm)	30.85	105.15
T_3(15 ppm)	32.80	98.64
T_4(20 ppm)	34.85	97.40
T_5(control)	33.25	99.05
CD 5%	2.82	2.69
SEm	0.91	0.87

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