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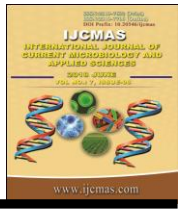

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Effect of Foliar Spray of Micro-Nutrients on Pigment Content in Pot Mum Cultivars Ajina Purple and Dolly White

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A B S T R A C T

A pot experiment was conducted during September-2010 to February-2011 at College of Horticulture, Mandsaur (RVSKVV Gwalior), to find out the response of pot mum cultivars Ajina Purple and Dolly White to micro-nutrient sprays viz. Fe, Zn, Mn and B. the different micro-nutrienst alone and in combinations were sprayed to find out the best micro-nutrient treatment for these cultivars in terms of pigment content. The cv. Ajina Purple with 0.4% Fe recorded highest chlorophyll content (2.23 mg/g and 2.18 mg/g) before and after flowering respectively. The highest anthocyanin content 0.86 mg/g was recorded by Fe 0.4% and Zn 0.4% with cv. Ajina Purple and the highest leuco- anthocyanin content 0.82 mg/g was observed with cv. Dolly White.

Introduction

Chrysanthemum has been considered number one among the major pot crops and year round pot mums are now grown all over the world. Pot mum production has become the most profitable form of commercial chrysanthemum growing. Economy of time, space and material has made this style of growing very promising. An excellent range of colour, form, long lasting quality of blooms and ease of handling make them most popular. The bright, colourful flowers provide a wide range of sales opportunities to garden centres, supermarkets, home improvement stores and mass market outlets. Availability of micro-nutrients from soil to the plants is affected by type of soil, ambient temperature, moisture condition of soil (Rao, 2005), soil pH, and nature of soil, so foliar application is more effective for nutrient uptake of plants.

Micronutrients play many vital roles in plant nutrition uptake, but most of them are used in the functioning of a number of enzyme systems. However, there is considerable variation in the specific functions of the various micronutrients in plants and in microbial growth processes. Nowadays micronutrients are gradually gaining momentum among the flower growers because of their beneficial nutrient support and at the same time to ensure better harvest and return (Balakrishnan *et al.*, 2007). Micronutrients assist in nitrogen assimilation and synthesis of protein (Kumar *et al.*, 2003). Micronutrients

have a great bearing in influencing the yield attributes and flower production. Application of micronutrients is found to enhance the foliage and flower production. Micronutrients activate several enzymes and are involved in various physiological activities (Sinha *et al.*, 1999).

Materials and Methods

One healthy rooted cutting was planted in one pot. Before planting the roots were dipped in carbendazim 3 g/litre of water, for 10 minutes. Plants were planted at the centre of the pots, watering was done and pots were kept in shade. Micro-nutrients (Fe-EDTA, Zn-EDTA, Mn-EDTA and Borax) spray was done three times. The first stage was active vegetative growth (30 DAP), the second at bud initiation stage (50 DAP) and the third at peak flowering (90 DAP).

Determination of chlorophyll content

Prepare 80% acetone. Grind the known weight pieces of plant material (avoid mid ribs) in mortar and pestle using 5 ml of 80% acetone. Filter the homogenate in 25 ml volumetric flask by using Whatman filter paper grade-1. Wash out the homogenate 3-4 times with 5 ml of 80% acetone each time. Grind the tissue once again with minimum quantity of acetone if required as it helps in complete extraction of plant pigments. Centrifuge the tubes (2000 rpm) for 10 minutes. Collect the supernatant in 25 ml volumetric flask and make up the volume with 80% acetone.

Record the absorbance of supernatant at two different wavelengths (663 and 645 nm) using spectrophotometer by keeping 80% acetone as blank.

The amount of total chlorophyll is determined using the following formula given by Arnon (1949).

$$\text{Chlorophyll “a”} = \frac{12.7\,(A\ 663) - 2.69\,(A\ 645)}{a \times 1000 \times w} \times V$$

$$\text{Chlorophyll “b”} = \frac{22.9\,(A\ 645) - 4.68\,(A\ 663)}{a \times 1000 \times w} \times V$$

Total Chlorophyll = Chlorophyll “a” + Chlorophyll “b”

Where,
A: optical density
a: length of light path in cell
w: weight of sample (g)
V: volume of solution

Determination of anthocynin pigment

Grind a known weight of fresh plant material in alcohol then filter or centrifuge and collect the extract. Pipette 1 ml of the alcohol extract into the test tube and add 3ml or HCI in aqueous methanol. Add 1 ml of anthocyanin reagents to the samples. Prepare the blank in the same manner by adding 1ml of methanol-HQ instead of anthocyanin reagent. After 15 min of incubation in the dark, measure the absorbance at 525nm against the blank. Calculate the amount of anthocyanins present in the sample from a standard curve prepared with cyanin hydrochloride.

Determination of leuco-anthocynin pigment

Grind a known weight of tissue in methanol or ethanol filter or centrifuge and collect the supernatant. Pipette 1ml of the extract in to a test tube. Reduce the volume to 0.5 ml on a hot water bath so that the sample does not contain more than 0.5 ml of methanol or ethanol. Add 0.5 ml of water and 10 ml of leuco-anthocyanin reagent and mix thoroughly. Heat the tubes in a water bath at 97± 1^0C for 3min without covering the tubes. Cover the tubes with glass stoppers and

continue heating for a total of 40 min. Cool under a running tap. Maintain the blank similarly with the extract but without heating. Measure the absorbance at 550 nm a colorimeter and express the results as A_{550} values.

Procedure follows in determination Leaf nutrient content

Materials required

Dried plants, H_2SO_4, $HClO_4$, double distilled water, willymill, funnels, Whatman filter papers grade 1, volumetric flasks, measuring cylinders, pipettes, AAS (Hitachi Z-2300) etc.

Procedure

Dried plants were harvested. The root portion was removed and plants were dried in hot air oven on 75 ^{0}C for 24 hours. Dried samples were ground in willymill and dried powder was used for analysis. 1.0 g dried powder was digested in di-acid mixture 15 ml (H_2SO_4:$HClO_4$, 4:1) at the temperature of 350-400^{0}C using Kel-plus KES-12 furnace. After complete digestion the digested extract was diluted with double distilled water up to 50 ml. Diluted extract was analyzed for micro-nutrients on AAS (Hitachi Z-2300).

Results and Discussion

Chlorophyll content before and after flowering

It can be observed that the mean total chlorophyll content before flowering was more in V1 (1.19 mg/g) in comparison to V2 (1.84mg/g), whereas after flowering it was more in V2 (1.77 mg/g) than V1 (1.76 mg/g). Among the different micronutrient sprays under studyT1 sprays recorded the highest total chlorophyll content before and after flowering it was (2.20mg/g and 2.12 mg/g) respectively followed by T2 (2.19mg/g and 2.07mg/g). While the total lowest chlorophyll content (1.00 mg/g and 0.69 mg/g) respectively recorded by T11 (Control). The treatment combination T1V1 spray recorded the highest total chlorophyll content before and after flowering (2.23 mg/g and 2.18mg/g) followed by T2V1 (2.21mg/g) and T2V2 (2.12 mg/g) before and after flowering. The total lowest chlorophyll content before and after flowering was recorded by T11V2 (0.84mg/g and 0.65 mg/g). This is an important consideration since iron is associated with chlorophyll formation and is a constituent of several plant compounds and enzymes as they are involved in energy producing and utilizing process in the plant and is important in many redox reactions (Pratap *et al.*, 2005). This may be due to indirect role of iron in chlorophyll Bio-synthesis iron play a very important role in chlorophyll synthesis and pigmentation. The above findings are in close agreement with finding of Balakrishnan *et al.*, (2007) in marigold.

Anthocynin and leuco anthocynin

It can be observed from table 1 that the mean anthocyanin content in petals of V1 was 0.78mg/g and leuco anthocynin content was V2 (0.71 mg/g). The highest anthocynin content (0.86 mg/g) was recorded by T1&T3 followed by T2 (0.83mg/g) whereas total leuco-anthocynin content 0.82mg/g was recorded by T3 followed by T1 (0.79mg/g). The lowest anthocynin and leuco anthocynin content was recorded by T11 (0.66mg/g and 0.55mg/g) respectively. Increase in pigment content due to the micronutrients (Fe and Zn) activate several enzymes (catalase, peroxidase, alcohol, dehydrogenase, carbonic dehydrogeniase and tryptophan synthatase, etc.) and involved themselves in chlorophyll synthesis and various physiological activities (Kumar and Haripriya, 2010).

Table.1 Effect of Micro-nutrient on chlorophyll, anthocynin and leuco-anthocynin content in pot-mum

Treatment	Chlorophyll content in leaves before flowering (mg/g)			Chlorophyll content in leaves before flowering (mg/g)			Anthiocyanin content (mg/g)		Leuco-Anthiocyanin content (mg/g)	
	V1	V2	Mean	V1	V2	Mean	V1		V2	
T1 (Fe-0.4%)	2.23	2.16	2.20	2.18	2.06	2.12	0.86		0.79	
T2 (Fe-0.8%)	2.21	2.17	2.19	2.02	2.12	2.07	0.83		0.74	
T3 (Zn-0.4%)	1.96	1.98	1.97	1.88	1.90	1.89	0.86		0.82	
T4 (Zn-0.8%)	2.19	2.11	2.15	2.11	2.03	2.07	0.81		0.75	
T5 (Mn-0.4%)	1.91	1.87	1.89	1.86	1.81	1.84	0.78		0.76	
T6 (Mn-0.8%)	1.94	1.90	1.92	1.53	1.81	1.67	0.71		0.68	
T7 (Borex-0.2%)	1.94	1.89	1.92	1.81	1.82	1.81	0.80		0.69	
T8 (Borex-0.4%)	1.77	1.63	1.70	1.65	1.59	1.62	0.78		0.64	
T9 (Fe+Zn+Mn-0.4%+Borex-0.2%)	1.82	1.84	1.83	1.74	1.78	1.76	0.75		0.73	
T10 (Fe+Zn+Mn-0.8%+Borex-0.4%)	1.94	1.88	1.91	1.92	1.83	1.88	0.78		0.66	
T11 (Control)	1.16	0.84	1.00	0.72	0.65	0.69	0.66		0.55	
Mean For factor	**1.91**	**1.84**	**1.88**	**1.76**	**1.77**	**1.76**	**0.78**		**0.71**	
Factor	**V**	**T**	**V*T**	**V**	**T**	**V*T**	**V**	**T**	**V**	**T**
S.E (m)+	0.03	0.08	0.11	0.04	0.09	0.13	0.007	0.017	0.007	0.015
CD at 5%	NS	0.24	NS	NS	0.26	NS	0.021	0.050	0.019	0.045

V1= Ajina Puple, V2= Dolly White

Table.2 Effect of micro-nutrient on leaf nutrient content

Treatment	Zn			Fe			Mn		
	V1	V2	Mean	V1	V2	Mean	V1	V2	Mean
T1 (Fe-0.4%)	1.67	1.28	1.48	1.69	1.30	1.50	0.79	0.79	0.79
T2 (Fe-0.8%)	1.83	1.73	1.78	1.97	1.76	1.86	0.76	0.74	0.75
T3 (Zn-0.4%)	1.70	1.64	1.67	1.72	1.66	1.69	0.67	0.82	0.74
T4 (Zn-0.8%)	1.71	1.66	1.69	1.72	1.68	1.70	0.81	0.75	0.78
T5 (Mn-0.4%)	1.46	1.45	1.46	1.45	1.45	1.45	0.80	0.78	0.79
T6 (Mn-0.8%)	1.01	1.64	1.33	0.99	1.61	1.30	0.71	0.68	0.69
T7 (Borex-0.2%)	1.18	1.31	1.25	0.98	1.31	1.14	0.80	0.69	0.75
T8 (Borex-0.4%)	0.97	1.27	1.12	0.98	1.26	1.12	0.78	0.64	0.71
T9 (Fe+Zn+Mn-0.4%+Borex-0.2%)	1.49	1.53	1.51	1.66	1.51	1.75	0.75	0.73	0.74
T10 (Fe+Zn+Mn-0.8%+Borex-0.4%)	1.55	1.67	1.61	1.41	1.37	1.98	0.78	0.66	0.72
T11 (Control)	0.93	0.95	0.94	0.92	0.94	0.93	0.66	0.55	0.60
Mean For factor	**1.41**	**1.47**		**1.41**	**1.44**		**0.75**	**0.71**	
Factor	V	T	V*T	V	T	V*T	V	T	V*T
S.E (m)+	0.05	0.11	0.15	0.05	0.12	0.16	0.01	0.03	0.04
CD at 5%	NS	0.33	NS	NS	0.33	NS	0.03	0.08	0.11

V1= Ajina Puple, V2= Dolly White

Iron plays a very important role in chlorophyll synthesis and pigmentation (Naik *et al.*, 2008).

Leaf nutrient content

It can be observed from table 2 above finding that mean Zn and Fe content was more in V2 (1.47 ppm and 1.44 ppm) in comparison to V1 (1.41 ppm) whereas the mean Mn content was more in V1 (0.75 ppm) then V2 (0.71ppm) Among the different nutrient sprays under study T2 recorded the highest Zn and Fe (1.78 ppm and 1.86 ppm) followed by T3 which were recorded (1.67 ppm and 1.69 ppm) while the highest Mn content under different micro-nutrient sprays T1 and T5 contain 0.79 ppm. The lowest Zn, Fe and Mn content in leaves was recorded by T11 (0.94 ppm Zn, 0.93ppm Fe and 0.60 ppm Mn), under the different treatment combination T2V1 was recorded the highest Zn and Fe content (1.83 ppm1.97 ppm) followed by T2V2 (1.73ppm and 1.76 ppm) and T4V1 content highest Mn 0.81 ppm.

The lowest Zn and Fe content was recorded by treatment combination T11V1 (0.93 ppm and 0.92 ppm) whereas the lowest Mn content was recorded by T11V2 (0.55 ppm). Micro-nutrients (Fe, Zn and Mn) are co-factor for several enzymes (Cytochromes, Peroxidase, Catalase, Ferodoxin, Alcohol dehydrogenase, Arginase, phosphotransferase, carbonic anhydrase and carboxy peptidase, etc.) and involved themselves in chlorophyll synthesis and various physiological activities. These results are in accordance with the finding of Pratap *et al.*, (2005).

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