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Original Research Article

Flowering and Physiological Response of African Marigold (*Tagetus erecta* L.) cv. Pusa Basanthi Gainda to Nutrients and *Piriformospora indica* (PGPRE)

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A B S T R A C T

The present investigation on African marigold (*Tagetus erecta* L.) cv. Pusa Basanthi Gainda was conducted during Rabi season of 2016-2017 under agro-climatic condition of College of Horticulture, Anantharajupeta, Y.S.R Kadapa Dt. The experiment was conducted to study the influence of nutrients and *Piriformospora indica* on flowering and physiology of African marigold (*Tagetus erecta* L.) cv. Pusa Basanthi Gainda. The investigation was designed in Randomized Block Design, replicated thrice with 9 treatments. The results revealed that, the treatment T_2 recorded significantly maximum flower diameter (9.27 cm), fresh weight of total flowers (394.47 g plant^{-1}), flower yield (29.06 t ha^{-1}), plant biomass (34.27 g plant^{-1}) and chlorophyll a, b, total chlorophyll content (0.181, 0.184 and 0.365 mg g^{-1} fresh wt.). However, significantly higher number of flowers plant^{-1} (53.62) and leaf area (45.75 cm^2) was recorded in T_3 and T_7. Whereas, the treatment combination T_5 recorded maximum crop growth rate (2.78 g m^{-2} day^{-1}), relative growth rate (0.022 g day^{-1}) and net assimilation rate (0.047 g m^{-2} day^{-1}). It was concluded that the treatment combinations T_2 and T_5 was found better for enhancing flowering and physiological attributes, respectively.

Keywords

African marigold, Nutrients, *Piriformospora indica*, Flowering and physiological attributes

Introduction

Marigold is a free blooming ornamental crop and used as a loose flower that is gaining popularity on account of its easy culture, wide adaptability, and increasing demand in National and International flower trade (Ahmad *et al.*, 2011). It is the most important traditional flower crop of India. It is one of the most important commercial flower crop grown all over the world and in India as well, accounting for more than half of the Nation's loose flower production (Raghava, 2000). Marigold ranks first among the loose flowers followed by chrysanthemum, jasmine, tuberose, crossandra and barleria (Bhattacharjee, 2003). In India, marigold is grown on commercial scale in about 56.04 thousand hectares with a production of 9.15 thousand MT. Andhra Pradesh is one of the leading states with an extent of 5.55 thousand ha area and annual flower production of 43.10 thousand MT (Anonymous, 2015-16).

Nutrients are essential elements required by the plants for growth and development. Nitrogen is an essential part of nucleic acid

and plays a vital role in promoting the plant growth. Similarly, an adequate supply of phosphorus is associated with rapid and vigorous start to plant, helping to establish seedling quickly, stimulates flowering and decrease lodging tendency of plant since phosphorus is a constituent of chlorophyll and is involved in many physiological processes including cell division, development of meristematic tissue, photosynthesis, metabolism of carbohydrates, fats and proteins (Acharya and Dashara, 2004). In addition, Moreira *et al.,* (2010) illustrated that phosphorus and nitrogen are the most limiting factors for plant growth and also required for AMF and Rhizobia symbiosis. Nitrogen, P and K also plays many different roles in plants for photosynthesis, regulates the opening and closing of stomata.

Potassium triggers activation of enzymes and is essential for production of Adenosine Triphosphate (ATP). Even though marigold cultivated on a large scale, its nutrient requirements have not been assessed for Rayalaseema region of Andhra Pradesh. In the absence of precise recommendations, the growers are following nutrient schedules of their own, which results in improper nutrition to the crop. This ends up with improper balance in plants and is considered to be a major factor contributing to low yields which poses a serious problem in flower production. Hence, the nutrient supply should be adjusted to the specific requirements of the plants during various stages of growth to attain maximum level of yields.

Piriformospora indica AM fungi – like fungus, showed prominent positive influence on a wide range of plants of agriculture, forestry and flori-horticultural importance. Fungus has a wide host range of monocots and dicots including legumes, terrestrial orchids (*Dactylorhiza maculata*) and members of the bryophytes (*Aneura pinguis*). The fungus showed potential as an agent for biological control of disease against soil-borne root pathogens. ^{32}P experiments suggest that this fungus is important for phosphorus acquisition by the roots, especially in the arid and semi-arid regions. Mycelium could utilize a wide variety of inorganic and organic phosphate chemicals and produced acid phosphatases at the tip of the hyphae (Singh *et al.,* 2003a, b). However, very little experimental work has been done on the nutritional requirements of marigold particularly nitrogen, phosphorus, potassium and in combination with *Piriformospora indica* (PGPRE) in this important flowering crop under the tropical conditions of semi-arid zone of Southern Andhra Pradesh. Because of the absence of relevant information on these aspects, the present investigation was conceived and conducted with N, P and K (RDF) at different levels along with *Piriformospora indica* (PGPRE) to arrive at a feasible nutrient schedule under the prevailing agro-climatic conditions of the Rayalaseema zone in Andhra Pradesh.

Materials and Methods

The present investigation on African marigold (*Tagetus erecta* L.) cv. Pusa Basanthi Gainda was conducted during Rabi season of 2016-2017 under agro-climatic condition of College of Horticulture, Anantharajupeta, Y.S.R Kadapa Dist. The experiment was conducted to study the influence of nutrients and *Piriformospora indica* on flowering and physiology of African marigold (*Tagetus erecta* L.) cv. Pusa Basanthi Gainda. The investigation was laid out in Randomized Block Design, replicated thrice. The experiment consisting of 9 treatments viz., T1- 100 % RDF + *Piriformospora indica* inoculated to seeds, T2 - 75% RDF + *Piriformospora indica* inoculated to seeds, T3- 50 % RDF +

Piriformospora indica inoculated to seeds, T4- 100 % RDF + *Piriformospora indica* inoculated to seedling roots at the time of transplanting, T5- 75% RDF + *Piriformospora indica* inoculated to seedling roots at the time of transplanting, T6-50 % RDF + *Piriformospora indica* inoculated to seedling roots at the time of transplanting, T7-75% RDF + *Piriformospora indica* inoculated before transplanting, T8- 75% RDF + *Piriformospora indica* inoculated after pinching (40 days after transplanting), T9-Control.

After ploughing and digging, the land was brought to fine tilth. All weeds were completely removed from the field. All the stubbles of previous crop were removed from the field and burnt. The required numbers of plots (27) were prepared of size (2.00 m x 2.40 m) with bunds of 30 cm between plots. The length of experimental field is 25.20 m and width was 7.50 m. Well decomposed farmyard manure was applied uniformly to all the experimental plots at 25 t ha-1 and mixed well. Nitrogen (200 kg ha-1), phosphorus (80 kg ha-1) and potassium (80 kg ha-1) (as per Dr.Y.S.R.H.U, Andhra Pradesh recommendation) were applied.

The entire quantity of phosphorus and potash and 50 per cent of nitrogen were applied as basal dose and remaining 50 per cent nitrogen was applied as a top dressing at three weeks after transplanting in the main field. As per the treatments, initially some seeds were sown separately in the nursery without PGPRE treatment (for control and other treatments purpose) and again few seeds were treated with PGPRE (*Piriformospora indica*) (for treating 1 kg seed, require 200-250 g *Piriformospora indica*. Moist the seeds with 5 per cent jaggery (gur) in water solution and then add and mix *Piriformospora indica* culture powder. The gur (jaggery) solution makes the seed sticky and helps in coating of seeds with the PGPRE powder) and then seed is sown separately in another nursery.

Thirty-days-old healthy seedlings of uniform growth were transplanted. Transplanting was done in the evening on 29-11-2016 and light irrigation was given immediately after planting. For root inoculation, prepared a slurry/ thick solution by mixing *Piriformospora indica* formulation with plain water. Dip the roots in solution overnight and plant them in the next day, the quantity of solution should be sufficient enough to cover with *Piriformospora indica* solution. Solution is prepared by mixing 75-100 g *Piriformospora indica* in 100 ml water. Immediately after transplanting, a light irrigation was given to the crop for better establishment of the seedlings in the field.

Piriformospora indica was also applied after pinching (40 days after transplanting). For 1 sq.m area, 100 g *Piriformospora indica* was used before transplanting and at the time of pinching. Necessary plant protection measures were followed to prevent pest incidence. At initial stages of growth, chlorpyriphos @ 2-3 ml litre-1 of water was sprayed to control *Spodoptera litura*, while no disease incidence was noticed during investigation period.

For recording observations, five plants were selected per each plot at random and were labelled properly by indicating treatments. The data on physiological traits were recorded with the procedures adopted in chlorophyll a, b and total chlorophyll (Hiscox and Israelsta, 1979), Crop growth rate (Radford, 1967), Relative growth rate (Watson, 1952) and Net assimilation rate (Williams, 1946).The data were analyzed using the procedure outlined by Panse and Sukhatme (1985).

Results and Discussion

Flower diameter

Data on diameter of flower presented differed significantly due to the influence of nutrients and *Piriformospora indica* in African marigold cv. Pusa Basanthi Gainda (Table 1). Significantly maximum flower diameter was recorded when the marigold seeds are treated with the input T_2 (9.27 cm) which was on par with T_3 (9.19 cm) and T_1 (8.82 cm). Maximum flower diameter in the nutrient combination T_2 might be due to the fact that nitrogen and phosphorus being the most important constituent of proteins, amino acids, enzymes and co-enzymes are responsible for cell division and elongation. Similarly, phosphorus is associated with phosphorylation and production of ATP, a compound required to maintain equilibrium between biochemical and 50 enzymatic reactions in the plant, which might have resulted in increased flower diameter. The increased flower diameter with nitrogen application could be explained in the light of the fact that balanced application of nitrogen resulted in increased carbohydrate assimilation leading to increased vegetative growth. These carbohydrates once translocated to productive organs undergo hydrolysis and get converted into the reducing sugars which ultimately helped in increasing flower diameter. *Piriformospora indica* application also increased the flower diameter due to root growth which helped in better root development resulting in more absorption of water and mineral nutrients from soil and ultimately the flower diameter improved. The present finding corroborates with the reports of Joshi *et al.,* (2013) in marigold.

Total number of flowers plant^{-1}

The information made available in Table 1 indicated that significance response of marigold plants to different nutrient treatments was individually and in combination. It is clear from the data that all the treatments resulted in significant increase in number of flowers plant^{-1}. Significantly higher number of flowers plant^{-1} was recorded in T_3 (53.62) which was on par with T_2 (53.29) and which was followed by T_1 (49.44). This could be attributed to a higher C/N ratio and increased plant metabolism. The increased vegetative growth and balance C/N ratio could lead to increased synthesis of carbohydrate which ultimately promoted greater flowering. Similar results were also reported by Singh *et al.,* (2015) in marigold cv. Pusa Narangi Gainda.

Fresh weight of total flowers plant^{-1}

The results recorded on the fresh weight of total flowers plant^{-1} in response to the various nutrient treatments are furnished in Table 1. The information presented in the Table 1 has clearly demonstrated the significant influence of various treatments on the fresh weight of the total flowers plant^{-1}. The treatment combination T_2 resulted in highest fresh weight of total flowers plant^{-1} (394.47 g) which was followed by T_1 (262.08 g) and T_3 (207.99 g) which differ significantly with each other and independent from all other treatments. Since there was an increase in weight of single flower per plant due to increase in uptake of plant nutrients, it has in turn resulted in fresh weight of total flowers plant^{-1}. These results are in accordance with the findings of Kaushik *et al.,* (2013) in African marigold.

Flower yield

The data corresponding to this attribute was presented in Table 1. A perusal of the data indicated that marigold flower yield recorded was highest in T_2 (29.06 t ha^{-1}) and identified significantly superior, which was

followed by T_3 (26.66 t ha^{-1}) which was on par with T_1 (26.39 t ha^{-1}), T_5 (26.32 t ha^{-1}), T_6 (25.49 t ha^{-1}), T_8 (25.43 t ha^{-1}), T_7 (25.42 t ha^{-1}) and T_4 (25.30 t ha^{-1}).

The above findings with respect to flower attributes due to increased supply of major plant nutrients, which are required in larger quantities for the growth and development of plants. The application of nitrogen at optimum level attributed to acceleration in development of growth and reproductive phases. Moreover, higher content of nitrogen might have accelerated protein synthesis, thus promoting earlier floral primordial development. Thus, results are in conformity with the findings of Acharya and Dashora (2004) in African marigold. The increase in phosphorus is also found to be involved in the initiation of flower primordial formation leading to increase in size and number of flowers in African marigold. These results are in close agreement with the findings of Singh *et al.*, (2015) in marigold.

Leaf area

An examination of the data shows that leaf area was significantly influenced by various treatments (Table 2). Among various treatments, highest leaf area was recorded in T_7 (45.75 cm^2) and identified significantly superior, which was followed by T_3 (42.10 cm^2) which was on par with T_1 (39.27 cm^2). The increase in leaf number results in increase in leaf area (or) increase in leaf area can be attributed to increase in leaf number. The influence of *Piriformospora indica* and nutrients could influenced the production of more number of leaves which ultimately resulted in more leaf area. The higher increment of leaf area registered with *Piriformospora indica* inoculated plant could also be due to result of increased phosphorus uptake due to which biosynthesis processes are enhanced, determining a faster growth and development, which leads to a greater leaf area. This was supported by the findings of Aier *et al.,* (2015) in gladiolus.

Chlorophyll a, b and total chlorophyll

The data furnished in Table 2 recorded on chlorophyll ‘a’, ‘b’ and total chlorophyll content responded significantly to all the plant growth promoters. The perusal of the data presented in the Table revealed that, maximum chlorophyll ‘a’ content was recorded in T_2 (0.181 mg g^{-1} fresh wt.) which was on par with T_1 (0.179 mg g^{-1} fresh wt.), T_3 (0.178 mg g^{-1} fresh wt.), T_6 (0.178 mg g^{-1} fresh wt.) and T_7 (0.177 mg g^{-1} fresh wt.). Among all the treatments, highest chlorophyll ‘b’ content was recorded in T_2 (0.184 mg g^{-1} fresh wt.) and found significantly superior which was followed by T_1 (0.176 mg g^{-1} fresh wt.) and T_3 (0.156 mg g^{-1} fresh wt.). Higher total chlorophyll content was recorded in T_2 (0.365 mg g^{-1} fresh wt.) which was identified significantly superior to all other treatments which was followed by T_1 (0.355 mg g^{-1} fresh wt.) and T_3 (0.334 mg g^{-1} fresh wt.). The improved chlorophyll contents of leaves could be attributed to enhanced uptake of Mg, Fe and Cu in the presence of *Piriformospora indica* which are essential for synthesis of chlorophyll. The results are in consonance with earlier findings of Sajjad *et al.,* (2015) in gladiolus.

The ratio of chlorophyll ‘a’ to chlorophyll ‘b’ in the chloroplast is normally 3:1. It is known that the chlorophyll a to b ratio is higher in high-light growth conditions than in low - light growth conditions (*i.e.* more chlorophyll b in shade plants). Chlorophyll ‘b’ absorbs light at different wavelengths than chlorophyll ‘a’ and extends the range of light that could be used for

photosynthesis.This is further explained that, when there is higher total chlorophyll content and naturally higher the plant growth, higher rate of photosynthesis, more transpiration occur and hence the result for higher total chlorophyll content in the leaves. The amount of chlorophyll present had a direct relationship with the rate of photosynthesis because it is the pigment which is photoreceptive and is directly involved in trapping the light energy.

Plant biomass

The information made available in Table 3 indicated plant biomass varied significantly due to the influence of nutrients and *Piriformospora indica*. Marigold plants which received the treatment T_2 recorded higher plant biomass (34.27 g plant^{-1}) which was statistically on par with T_1 (33.99 g plant^{-1}) and T_3 (32.39 g plant^{-1}). This might be due to increased number of leaves plant^{-1} resulting in better photosynthesis and accumulation of photosynthates leading to more vigour. Increase in dry matter production due to enhanced uptake of macro and micronutrients, increased photosynthesis and sugar content in *Piriformospora indica* inoculated plants compared to uninoculated plants and increased number of leaves and leaf area which determines the photosynthetic efficiency of plants and dry matter production. These results are in conformity with findings of Ramana *et al.*, (2010) in French bean. The increase in fresh and dry weights in treated marigold plants may be related to increased root P uptake by the *Piriformospora indica.*

Table.1 Effect of RDF and *Piriformospora indica* (PGPRE) on floral attributes in African marigold cv. Pusa Basanthi Gainda

Treatments	Flower diameter (cm)	Total no. of flowers plant^{-1}	Fresh wt. of total flowers plant^{-1} (g)	Flower yield (t ha^{-1})
T_1 -100% RDF + *Piriformospora indica* inoculated to seeds	8.82	49.44	262.08	26.39
T_2-75% RDF + *Piriformospora indica* inoculated to seeds	9.27	53.29	394.47	29.06
T_3-50% RDF + *Piriformospora indica* inoculated to seeds	9.19	53.62	207.99	26.66
T_4-100% RDF + *Piriformospora indica* inoculated to seedling roots at the time of transplanting	8.50	46.28	194.83	25.30
T_5-75 % RDF + *Piriformospora indica* inoculated to seedling roots at the time of transplanting	8.68	43.63	190.61	26.32
T_6-50 % RDF + *Piriformospora indica* inoculated to seedling roots at the time of transplanting	8.72	41.56	193.87	25.49
T_7-75 % RDF + *Piriformospora indica*inoculated before transplanting	8.47	38.35	186.34	25.42
T_8- 75 % RDF + *Piriformospora indica* after pinching (40 days after transplanting)	8.33	36.55	186.61	25.43
T_9- Control	7.97	31.82	175.28	23.61
SEM ±	0.15	0.92	2.58	0.48
CD (*P*= 0.05)	0.46	2.78	7.82	1.46

Table.2 Effect of RDF and *Piriformospora indica* (PGPRE) on leaf area and chlorophyll content in African marigold cv. Pusa Basanthi Gainda

Treatments	Leaf area (cm^2)	Chlorophyll (mg g^{-1} fresh wt.)		
		Chlorophyll 'a'	Chlorophyll 'b'	Total chlorophyll
T_1 -100% RDF + *Piriformospora indica* inoculated to seeds	39.27	0.179	0.176	0.355
T_2-75% RDF + *Piriformospora indica* inoculated to seeds	34.32	0.181	0.184	0.365
T_3-50% RDF + *Piriformospora indica* inoculated to seeds	42.10	0.178	0.156	0.334
T_4-100% RDF + *Piriformospora indica* inoculated to seedling roots at the time of transplanting	37.83	0.174	0.132	0.306
T_5-75 % RDF + *Piriformospora indica* inoculated to seedling roots at the time of transplanting	37.52	0.172	0.125	0.299
T_6-50 % RDF + *Piriformospora indica* inoculated to seedling roots at the time of transplanting	32.65	0.178	0.149	0.327
T_7-75 % RDF + *Piriformospora indica*inoculated before transplanting	45.75	0.177	0.146	0.323
T_8- 75 % RDF + *Piriformospora indica* after pinching (40 days after transplanting)	38.24	0.175	0.134	0.309
T_9- Control	33.52	0.171	0.124	0.295
SEM ±	0.99	0.001	0.001	0.002
CD (*P*= 0.05)	3.01	0.004	0.005	0.006

Table.3 Effect of RDF and *Piriformospora indica* (PGPRE) on physiological attributes of African marigold cv. Pusa i Gainda

Treatments	Plant biomass (plant^{-1})	CGR (g m^{-2} day^{-1})	RGR (g day^{-1})	NAR (g m^{-2} day^{-1})
T_1 -100% RDF + *Piriformospora indica* inoculated to seeds	33.99	2.41	0.012	0.036
T_2-75% RDF + *Piriformospora indica* inoculated to seeds	34.27	2.48	0.019	0.027
T_3-50% RDF + *Piriformospora indica* inoculated to seeds	32.39	1.84	0.015	0.032
T_4-100% RDF + *Piriformospora indica* inoculated to seedling roots at the time of transplanting	30.40	2.04	0.009	0.036
T_5-75 % RDF + *Piriformospora indica* inoculated to seedling roots at the time of transplanting	32.24	2.78	0.022	0.047
T_6-50 % RDF + *Piriformospora indica* inoculated to seedling roots at the time of transplanting	30.20	2.38	0.012	0.035
T_7-75 % RDF + *Piriformospora indica* inoculated before transplanting	28.65	1.43	0.013	0.020
T_8- 75 % RDF + *Piriformospora indica* after pinching (40 days after transplanting)	28.19	1.35	0.008	0.017
T_9- Control	27.58	1.20	0.007	0.016
SEM ±	0.68	0.21	0.001	0.001
CD (*P*= 0.05)	2.05	0.66	0.003	0.001

Crop growth rate

Analysis of data corresponding to crop growth rate (CGR) is presented in Table 3. Crop growth rate was significantly influenced by various treatments applied. The plant growth promoter T_5 recorded significantly higher CGR (2.78 g m^{-2} day^{-1}) which was on par with T_2 (2.48 g m^{-2} day^{-1}), T_1 (2.41 g m^{-2} day^{-1}) and T_6 (2.38 g m^{-2} day^{-1}). The CGR is the proportion of plant biomass and time period of growth. A similar trend was also observed in the case of CGR also. Highest CGR was recorded in those plants which received the combination of RDF and *Piriformospora indica*. Concurrent results were also reported by Naik and Kumar (2015) in *Dendrobium* cv. Earsakul.

Relative growth rate

The data pertaining to relative growth rate as influenced by different treatments are furnished in Table 3. Among the treatments tried, significantly higher RGR was recorded in T_5 (0.022 g day^{-1}) which was on par with T_2 (0.019 g day^{-1}). Since the marigold plants were in active growth phase, it was significantly showing the unit increasing dry matter production (DMP). This might lead to increase in RGR. The result in the present study was parallel with the findings of Dhinesh (2009) in *Dendrobium* cv. Earsakul.

Net assimilation rate

Net assimilation rate varied significantly due to the influence of RDF and *Piriformospora indica*and and the data was presented in Table 3. The result revealed that, significantly higher NAR was recorded in T_5 (0.047 g m^{-2} day^{-1}) which was found significantly superior to all other treatments, which was followed by T_4 (0.036 g m^{-2} day^{-1}) which was on par with T_1 (0.036 g m^{-2} day^{-1}) and T_6 (0.035 g m^{-2} day^{-1}). Increase in the physiological parameters might be due to synergistic effect of nutrients and *Piriformospora indica* in the activation of enzymes, which is required for the CO_2 assimilation pathway which increases the plant height, number of leaves and leaf area which in turn might have participated in photosynthesis and contributed to increase in the plant biomass, CGR, RGR and NAR. The results are in accordance with the findings of Sirisha *et al.,* (2016) in gladiolus cv. Arka Amar.

The maximum physiological attributes might be due to the treated plants showed pronounced growth relative to the non-inoculated control. Similar reports of increased growth on inoculation with *Piriformospora indica* have been observed for *Spilanthes calva* and *Withania somnifera* (Rai *et al.,* 2001).

Varma *et al.,* (1999) reported an increase in plant height, fresh and dry biomass and larger leaf area in micro propagated *Artemisia annua*, *Bacopa monnieri* and tobacco. The differences in growth observed between treated and control plants were suggested to be caused by greater absorption of water and nutrients due to extensive colonization of roots by *Piriformospora indica* (Rai *et al.,* 2001). Such plants show enhanced P uptake resulting in an increase in fresh and dry weights (Sudha *et al.,* 1998).

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