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Influence of nutrients and *Piriformospora indica* on growth and biochemical attributes of african marigold cv. Pusa basanthi Gainda

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Abstract
The present investigation on African marigold (*Tagetes 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 growth and biochemical traits of marigold. The experiment was laid-out in Randomized Block Design, replicated thrice and consisting of 9 treatments. The results revealed that, taller plant (58.29 cm), higher leaf count (105.20 plant^{-1}), early time to full flowering (59.55 days), longest root (19.21 cm), higher number of roots (127.33), root volume (39.33 m^3) and maximum root colonization of *Piriformospora indica* in marigold roots (75.09 per cent) was recorded in treatment combination T_2. However, carotenoid (31.32 mg g^{-1}) and total phenol content (76.55 mg g^{-1}) recorded was highest in the treatment T_5. While the treatment combinations *viz*., T_4 and T_3 recorded significantly maximum flavonoids (4.67 mg g^{-1}) and carbohydrates (6.82 mg g^{-1}), respectively.

Keywords: biochemical attributes, growth, flowering, marigold, *Piriformospora indica*

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) [2]. It is the most important traditional flower crop of India. Marigold (*Tagetes* spp. Linn.,) 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) [21]. Marigold belonging to family Asteraceae is a native of Central and South America, especially Mexico. Marigold ranks first among the loose flowers followed by chrysanthemum, jasmine, tuberose, crossandra and barleria (Bhattacharjee, 2003) [4]. 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) [3].

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 etc., (Acharya and Dashara, 2004) [1]. In addition, Moreira *et al*. (2010) [15] 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 it is 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) [24]. 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 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.

Material 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 Dt. Andhra Pradesh. The experiment was conducted to study the influence of nutrients and *Piriformospora indica* on growth, flowering and biochemical traits of African marigold (*Tagetes erecta* L.) cv. Pusa Basanthi Gainda. The experiment was laid-out in Randomized Block Design, replicated thrice. The trial consisting of 9 treatments *viz.,* T_1- 100 % RDF + *Piriformospora indica* inoculated to seeds, T_2 - 75% RDF + *Piriformospora indica* inoculated to seeds, T_3- 50 % RDF + *Piriformospora indica* inoculated to seeds, T_4- 100 % RDF + *Piriformospora indica* inoculated to seedling roots at the time of transplanting, T_5- 75% RDF + *Piriformospora indica* inoculated to seedling roots at the time of transplanting, T_6-50 % RDF + *Piriformospora indica* inoculated to seedling roots at the time of transplanting, T_7-75% RDF + *Piriformospora indica* application before transplanting, T_8- 75% RDF + *Piriformospora indica* application after pinching (40 days after transplanting), T_9-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 was applied as basal dose and remaining 50 per cent nitrogen was applied as 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 biochemical observations were recorded with the procedures adopted in carotenoids by **(**Srivasthava and Kumar, 2009), phenols (Malik and Singh,1980) flavonoids (Sahu and Saxena, 2013) [22], carbohydrate content (Hedge and Hofreiter, 1962) [7] and root colonization of marigold plants with *Piriformospora indica* (Giovannetti and Mosse, 1980) [6]. The data were analyzed using the procedure outlined by Panse and Sukhatme (1985) [19].

Results and Discussion

Plant Height

The data on influence of nutrients and *Piriformospora indica* on plant height of African marigold cv. Pusa Basanthi Gainda was presented in Table 1. Significantly tallest plant was recorded in T_2 (58.29 cm) which was on par with T_3 (56.54 cm), T_6 (52.21 cm), T_1 (50.71 cm) and T_4 (50.24 cm). It might be due to the combination of N,P and K along with AMF gave additive effect in increasing the plant height due to secretion of certain growth promoting substances like auxin, gibberellins, vitamins, and organic acids in soil with better nutrient uptake, photosynthesis, source-sink relationship, besides excellent physiological and biochemical activities due to presence of *Piriformospora indica* inoculation. These findings are in accordance with earlier reports of Kumar *et al.* (2013) in marigold and Hoseini *et al*. (2015) [8] in *Calendula officinalis*.

Number of leaves plant^{-1}

The data recorded on number of leaves $plant^{-1}$ as influenced by different plant growth promoters are presented in Table 1and indicated that the number of leaves of marigold cv. Pusa Basanthi Gainda was significantly influenced by different treatments in combination with PGPRE.Higher number of leaves $plant^{-1}$(105.20) was recorded in T_2 which was found significantly superior to remaining treatments which was followed by T_3 (92.58), which was on par with T_1 (90.21) and T_4 (89.55).

The maximum number of leaves plant^{-1} in treatment T_2might be due to easy absorption and translocation of nutrients by *Piriformospora indica* in roots, which promote protein synthesis from reserved carbohydrates leading to production of higher number of leaves plant^{-1} and other reason, might be due to increased nitrogen availability as it is a constituent of protein component of protoplast that increases the chlorophyll content in leaves. All these factors contribute to cell multiplication, cell enlargement and differentiation which could have resulted in better photosynthesis and ultimately exhibited better vegetative growth and increased number of leaves. These results are in consonance with earlier findings of Naik (2015) [16] in Heliconia cv. Local Yellow and Kumar *et al.* (2013) in gladiolus cv. White Prosperity.

Total number of branches at full bloom plant^{-1}

A perusal of the data furnished in Table 1 showed significant influence of different nutrient treatments on total number of branches plant^{-1}. Marigold cv. Pusa Basanthi Gainda plants treated with the input T_5produced higher number of total branches plant^{-1} at full bloom stage (99.43) which was on par with T_4 (99.07).

The increase in production of branches plant^{-1} might be due to highest level of nitrogen which promoted the auxiliary buds into new shoots. The significant increase in production of higher number of branches plant^{-1}could also be due to the influence of combination of NPK and *Piriformospora indica* resulted in increased availability of nitrogen, phosphorus as well as micronutrient like Zn which is a precursor of auxin, which improve the vegetative growth, dry matter accumulation and their partitioning towards the developing number of branches in African marigold. These results are in tune with the statement of Pushkar *et al*. (2011) [20]. Once endosymbiosis is established inside the roots, the *P. indica* fungus gets access to photo-assimilates and other plant nutrients, which further promotes colonization and proliferation of the fungus in roots, and thus significantly enhances plant growth (Oelmuller *et al*., 2009) [18].

Number of days for full flowering

The data available in Table 1 recorded on influence of RDF in combination with *Piriformospora indica* on number of days to full flowering responded significantly to all the nutrient combinations.Results showed that, flowering was advanced by T_2 (59.55 days) which was on par with T_1 (59.71 days) and T_4 (60.67 days). This might be due to the altered C:N ratio which helped in balanced management of vegetative as well as reproductive phases and promote early flowering. This was in accordance with the reports of Naik (2015) [16] in marigold.

Carotenoids

Data on this attribute are presented in Table 2. In fresh florets, carotenoid content varied significantly among various treatments. The highest carotenoid of 31.32 mg g^{-1}was recorded in T_5 which was significantly superior to all other treatments and was followed by T_1 (28.55 mg g^{-1}) which was on par with T_4 (28.15 mg g^{-1}) and T_2 (27.13 mg g^{-1}). The reason for the above finding could be due to application of nutrients and *Piriformospora indica* supply the plants with the essential elements for carotenoids, carotenoids formation in flowers associated with the conversion of chloroplasts in to chromoplasts. Chromoplasts formation commences with the disruption of grana thylokoids and disappearance of chlorophylls. Due to increase of chlorophylls, carotenoids also increased. In many chloroplasts compartments are formed in place of the grana in which carotenoids are synthesized. The above finding in the present investigation was supported by Kumar and Gupta (2013) [12] in gladiolus cv. Jessica and Sanghamitra *et al.* (2015) [23] in African marigold.

Total Phenols

Total phenol was significantly influenced by nutrients and *Piriformospora indica* (Table 2). Maximum total phenol content of 76.55 mg g^{-1} was recorded in T_5 which was on par with T_6 (75.28 mg g^{-1}) and T_2 (74.03 mg g^{-1}). The presence of *Piriformospora indica* had resulted in stimulation of plant growth regulators which had influenced carbon partitioning in the plant, increasing the number of carbohydrate precursors required for the synthesis of phenolic compounds. These results are in conformity with findings ofKumar and Gupta (2013) [12] in gladiolus cv. Jessica.

Flavonoids

Flavonoid content varied significantly due to the influence of nutrients and *Piriformospora indica*. A critical examination of the data from table showed that, significantly highest flavonoid content was recorded in T_4 (4.67 mg g^{-1}) which was on par with T_3 (4.41 mg g^{-1}) and this was on par with T_5 (4.14 mg g^{-1}). Increasing the access to nitrogen elements and phosphorus in soil due to the activity of *Piriformospora indica* and due to increasing their attraction by plant has caused to increase nitrogen, phosphorus and probably caused increase in the initial substrate of related reactions by producing secondary metabolites such as flavonoids. Increasing the efficiency of nitrogen attraction due to increasing the nutrients and bio fertilizers in African marigold resulted in increasing the flavonoids. The present finding corroborates with the reports of El-Moniem *et al*. (2013) [5] in broccoli.

Total carbohydrates

A perusal of the data in Table 2 indicated that response of total carbohydrate content was significant to RDF and *Piriformospora indica*. It was observed in fresh leaves that highest content of carbohydrates was recorded in T_3 (6. 82 mg g^{-1}) which was on par with T_2 (6.39 mg g^{-1}), T_1 (6.18 mg g^{-1}), T_6 (5.99 mg g^{-1}) and T_5 (5.95 mg g^{-1}). Maximum carbohydrate content in treatment T_3 might be due to highest value of the total carbohydrates in the leaves was obtained by application of NPK and *Piriformospora indica* each increase the leaf area and leaf chlorophyll content, consequently the rate of photosynthesis process would be increased, as a result the percentage of total carbohydrate in the leaves could also be increased, total carbohydrates was increased as a result of applying bio-fertilizer alone or combined with chemical fertilizers. These findings are in accordance with findings of Hussein *et al.* (2011) in *Calendula officinalis*.

Root length

Analysis of data corresponding to length of root is presented in Table 3. Root length varied significantly among various plant growth promoters tried. Significantly longest root of 19.21 cm was recorded in T_2 which was on par with T_4 (18.37 cm), T_1 (16.93 cm) and T_5 (16.92 cm). The reason could be due to that the *Piriformospora indica* made the P available to the plants which in turn increase the length of the roots due to the influence of P. It might also due to the root endophyte inoculation which has the potential to add nitrogen and phosphorus to crop growth through associative symbiosis and increased production of growth hormones like NAA, GA and cytokinins (Varma *et al*., 1999; Singh *et al.*, 2000) [29]. These phytohormones might have caused morphological change in length of roots, thereby causing an increase in uptake of nutrients resulting in better growth. *Piriformospora indica* colonized maize plants showed an increased biomass production, root length and root number compared to the non-colonized plants (Kumar *et al*., 2014).

Number of roots plant^{-1}

The data recorded on number of roots plant^{-1} as influenced by different treatments is presented in Table 3 indicated that the number of roots was significantly influenced by different dosages of RDF and *Piriformospora indica*. Among various inputs tried, significantly higher root count plant^{-1} was recorded in T_2 (127.33) which was on par with T_1 (113.95), T_3 (109.53) and T_5 (108.89).

The phosphorus nutrient in the early stages of growth is beneficial for producing more number of roots plant^{-1}. The plants having higher number of shoots and beneficial effects of *Piriformospora indica* enhanced better root system which in turn helps in rapid growth of the plant and ultimately plants are having maximum number of roots plant^{-1}. These results are in conformity with the findings of Naik and Kumar (2015) [16] in *Dendrobium* cv. Earsakul. Further, it was found that *P. indica* produces auxin IAA which is known for its stimulatory role in plant root growth (Sirrenberg *et al.*, 2013) [27]. *P. indica* has been reported to interfere with ethylene signaling in plants where, it promotes the plant root growth (Khatabi *et al.*, 2013) [10].

Root volume

A critical examination of the data revealed that, the highest root volume was recorded in T_2 (39.33 m^3) and found significantly superior, which was followed by T_3 (27.66 m^3) and this was on par with T_1 (26.60 m^3), T_7 (26.33 m^3), T_6 (25.60 m^3), T_5 (24.23 m^3) and T_8 (22.66 m^3). The treatment 75% RDF + *Piriformospora indica* inoculated to seeds (T_2) recorded highest number of roots and root length plant^{-1} in the earlier finding and this might be the reason for highest root volume.

Root colonization of *Piriformospora indica*

The data on this trait are presented in Table 3. A critical examination of the data revealed that, significantly higher colonization of marigold root was observed in T_2 (75.09 per cent) which was on par with T_3 (74.40 per cent), T_1 (70.84 per cent) and T_4 (67.17 per cent). Enhanced root production, root volume and root colonization of *P. indica* was observed in treatment combination involving *P. indica*. The positive influence of *P. indica* for the above root parameters was clearly evident from this study. In the present study, *P. indica* had a positive effect on root parameters, which confirm with the observation of Naik and Kumar (2015) [16] in *Dendrobium* cv. Earsakul. Higher temperature is also the main reason for higher root colonization. It was reported that *P. indica* at higher temperature (25-35 ^{0}C) resulted in higher mycelia growth (Varma *et al*., 1999) [29] and the higher temperature prevailing in the experimental site during the period of investigation (29.25 to 35.95 ^{0}C) might be the possible reason for higher root colonization in the host plants.

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

Treatments	Plant height (cm)	Number of leaves plant^{-1}	Total number of branches plant^{-1}at full bloom	No. of days for full flowering
T_1 -100% RDF + *Piriformospora indica* inoculated to seeds	50.71	90.21	97.76	59.71
T_2-75% RDF + *Piriformospora indica* inoculated to seeds	58.29	105.20	97.09	59.55
T_3-50% RDF + *Piriformospora indica* inoculated to seeds	56.54	92.58	76.16	62.05
T_4-100% RDF + *Piriformospora indica* inoculated to seedling roots at the time of transplanting	50.24	89.55	99.07	60.67
T_5-75 % RDF + *Piriformospora indica* inoculated to seedling roots at the time of transplanting	49.83	84.61	99.43	62.22
T_6-50 % RDF + *Piriformospora indica* inoculated to seedling roots at the time of transplanting	52.21	82.89	75.04	65.20
T_7-75 % RDF + *Piriformospora indica* inoculated before transplanting	48.35	85.13	71.51	66.43
T_8- 75 % RDF + *Piriformospora indica* after pinching (40 days after transplanting)	45.21	65.02	71.04	68.85
T_9- Control	45.17	59.42	63.04	69.92
SEM ±	2.76	1.54	0.91	0.43
CD (P= 0.05)	8.31	4.65	1.32	1.32

Table 2: Effect of RDF and *Piriformospora indica* (PGPRE) on biochemical attributes in African marigold cv. Pusa Basanthi Gainda.

Treatments	Carotenoids (mg g^{-1})	Total phenols (mg g^{-1})	Flavonoids (mg g^{-1})	Carbohydrates (mg g^{-1})
T_1 -100% RDF + *Piriformospora indica* inoculated to seeds	28.55	65.67	3.28	6.18
T_2-75% RDF + *Piriformospora indica* inoculated to seeds	27.13	74.03	2.56	6.39
T_3-50% RDF + *Piriformospora indica* inoculated to seeds	23.08	64.14	4.41	6.82
T_4-100% RDF + *Piriformospora indica* inoculated to seedling roots at the time of transplanting	28.15	69.96	4.67	5.61
T_5-75 % RDF + *Piriformospora indica* inoculated to seedling roots at the time of transplanting	31.32	76.55	4.14	5.95
T_6-50 % RDF + *Piriformospora indica* inoculated to seedling roots at the time of transplanting	25.11	75.28	3.43	5.99

T_7-75 % RDF + *Piriformospora indica* inoculated before transplanting	18.43	68.56	2.26	4.89
T_8- 75 % RDF + *Piriformospora indica* after pinching (40 days after transplanting)	18.28	53.89	2.99	4.84
T_9- Control	15.44	44.60	1.45	4.31
SEM ±	0.89	1.47	0.17	0.30
CD (*P*= 0.05)	2.71	4.47	0.51	0.93

Table 3: Effect of RDF and *Piriformospora indica* on root parameters in African marigold cv. Pusa Basanthi Gainda.

Treatments	**Root length (cm)**	**No. of roots plant^{-1}**	**Root volume (m^3)**	**Root colonization of *Piriformospora indica* (%)**
T_1 -100% RDF + *Piriformospora indica* inoculated to seeds	16.93	127.33	26.60	70.84
T_2-75% RDF + *Piriformospora indica* inoculated to seeds	19.21	113.95	39.33	75.09
T_3-50% RDF + *Piriformospora indica* inoculated to seeds	15.28	109.53	27.66	74.40
T_4-100% RDF + *Piriformospora indica* inoculated to seedling roots at the time of transplanting	18.37	99.53	19.90	67.17
T_5-75 % RDF + *Piriformospora indica* inoculated to seedling roots at the time of transplanting	16.92	108.89	24.23	61.78
T_6-50 % RDF + *Piriformospora indica* inoculated to seedling roots at the time of transplanting	16.21	102.40	25.60	63.21
T_7-75 % RDF + *Piriformospora indica* inoculated before transplanting	14.55	97.65	26.33	57.07
T_8- 75 % RDF + *Piriformospora indica* after pinching (40 days after transplanting)	14.05	96.53	22.66	57.76
T_9- Control	12.32	73.53	16.53	0.00
SEM ±	0.77	6.94	1.86	3.63
CD (*P*= 0.05)	2.33	20.98	5.63	10.97

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