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Research Article

Influence of Nutrients and *Piriformospora indica* (PGPRE) on Growth and Biochemical Attributes of African Marigold (*Tagetus erecta* L.) cv. Pusa Basanthi Gainda

K. Noorjahan*, M. Raja Naik[1]

*[1]College of Horticulture (Dr. YSRHU), Anantharajupeta – 516 105, Andhra Pradesh, India

*Corresponding Author E-mail: naik_raja2006@rediffmail.com

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ABSTRACT

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. (Dr. Y.S.R.H.U). The experiment was conducted to study the influence of nutrients and Piriformospora indica on growth and biochemical attributes of African marigold. The investigation was designed in Randomized Block Design, replicated thrice with 9 treatments. The results revealed that, the treatment combination 75% RDF + Piriformospora indica inoculated to seedling roots at the time of transplanting (T_5) recorded significantly higher number of branches plant^{-1}at full bloom stage (99.43), maximum carotenoid (31.32 mg g^{-1}) andtotal phenol content (76.55 mg g^{-1}). Significantly longer plant at full bloom stage (58.29 cm) and higher number of leaves plant^{-1}(105.20) was recorded in plants treated with 75% RDF + Piriformospora indica inoculated to seeds (T_2). Maximum diameter of 14.56 mm and carbohydrate content (6.82 mg g^{-1}) was recorded in 50% RDF + Piriformospora indica inoculated to seeds (T_3). Whereas, the nutrient combination 100 % RDF + Piriformospora indica inoculated to seedling roots at the time of transplanting (T_4) resulted in maximum flavonoid content (4.67 mg g^{-1}).

Key words: *African marigold, nutrients, Piriformospora indica, Growth and Biochemical 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[2]. 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[19]. 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[3].

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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[1]. In addition, Moreira *et al*.[14], 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[23,24]. 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 (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.

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 Dist (Dr. Y.S.R.H.U). 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.*, 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 inoculated before transplanting, T_8- 75% RDF + *Piriformospora indica* inoculated 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 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 biochemical attributes *viz*., total carotenoide content[25], phenol content[13], flavonoids[20]. and carbohydeate content[5]. were also recorded. The data were analyzed using the procedure outlined by Panse and Sukhatme[17].

RESULTS AND DISCUSSION

Plant height (cm) at full bloom stage

The data on influence of nutrients and *Piriformospora indica* on plant height of African marigold cv. Pusa Basanthi Gainda was presented in Table 1. At full bloom stage, significantly highest plant height was recorded in 75 % RDF + *Piriformospora indica* inoculated to seeds (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 increase in vegetative growth by nitrogen application is because of the fact that nitrogen is an essential part of nucleic acid, which plays a vital role in promoting plant growth whereas, phosphorus is involved in many physiological processes including cell division, development of meristmatic tissues, photosynthesis, metabolism of carbohydrates, fats and proteins. 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* bio inoculation in African marigold. These findings are in accordance with earlier reports of Sharma *et al*.[22], in African marigold cv. Pusa Basanthi Gainda, Kumar *et al.*[9], in marigold and Hoseini *et al*.[6], in *Calendula officinalis*.

Stem diameter (mm) at full bloom

The data in Table 1 indicated that, stem diameter was significantly influenced by nutrients and *Piriformospora indica* in African marigold cv. Pusa Basanthi Gainda. At full bloom stage, highest stem diameter was recorded in 50 % RDF + *Piriformospora indica* inoculated to seeds (T_3) (14.56 mm) which was on par with T_2 (14.47 mm), T_5 (14.11 mm) and T_1 (13.63 mm). The possible reason for the above finding might be due the combined application of *Piriformospora indica,* FYM along with half nitrogen and phosphorus proved to be beneficial for robust growth of plant as compared to other treatments. The application of *Piriformospora indica* proved to be beneficial to solubilize phosphorus in soil and it also secrete growth substances like auxins, which stimulated the plant metabolic activities and photosynthetic efficacy leading to better growth and development of plant. Above results are in conformity with the findings of Kumar and Singh[11]. in china aster, Pushkar *et al.*[18], and Kumar *et al*.[12], in African marigold. Further the increased number of leaves plant^{-1} resulting in better photosynthesis and accumulation of photosynthates leading to more vigour and hence the result of maximum stem diameter.

Number of leaves plant^{-1} at 60 days after planting

The data recorded on number of leaves plant^{-1} as influenced by different treatments presented in Table 1 revealed that the number of leaves of marigold cv. Pusa Basanthi Gainda was significantly influenced by different treatments in combination with PGPRE.At 60 DAT, higher number of leaves plant^{-1} was recorded in 75 % RDF + *Piriformospora indica* inoculated to seeds (T_2) (105.20) which was found significantly superior and 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 agreement with the findings of Naik[15] in Heliconia cv. Local Yellow and Kumar *et al.*[10], in gladiolus cv. White Prosperity.

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

A perusal of the data presented in the 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 75% RDF + *Piriformospora indica* inoculated to seedling roots at the time of transplanting (T_5) developed 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} might 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*.[18]. The increased nitrogen nutrition may also have accelerated

the process of cell division and differentiation. The above results are corroborated with the findings of Sunitha and Hunje[26] in African marigold.

Once endosymbioses is established inside the roots, the *Piriformospora 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[16].

Carotenoids

Data on this attribute are presented in Table 2. In fresh florets, carotenoid content varied significantly among various treatments. The highest carotenoid was recorded in 75%RDF + *Piriformospora indica* inoculated to seedling roots at the time of transplanting (T_5) (31.32 mg g^{-1}) 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 Hussein *et al.*[7], in *Calendula officinalis*, Kumar and Gupta[9] in gladiolus cv. Jessica and Sanghamitra *et al.*[21], in African marigold. Further the *P. indica* colonized plants also had 70-100% more amount of carotenoids[8]. in rice.

Total phenols

Total phenol was significantly influenced by nutrients and *Piriformospora indica* are presented in Table 2. Maximum total phenol content of 76.55 mg g^{-1} was recorded in 75%RDF + *Piriformospora indica* inoculated to seedling roots at the time of transplanting (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 *P.indica* had resulted in stimulation of PGRS 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 of El-Moniem *et al.*[4], in broccoli and Kumar and Gupta[9] in gladiolus cv. Jessica.

Flavonoids

The data due to influence of nutrients and *Piriformospora indica* on flavonoids was presented in Table 2. Flavonoid content was significantly influenced by nutrients and *Piriformospora indica*. A critical examination of the data from table showed that, significantly highest flavonoid content was recorded in 100 % RDF + *Piriformospora indica* inoculated to seedling roots at the time of transplanting (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. Similar results were also obtained by El-Moniem *et al.*[4], in broccoli.

Total carbohydrates

A perusal of the data in Table 2 indicated that the total carbohydrate content was significantly influenced by different nutrients and *Piriformospora indica*. It was observed in fresh leaves that highest content of carbohydrates was recorded in 50 % RDF + *Piriformospora indica* inoculated to seeds (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 be also 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.* [7], in *Calendula officinalis*, Wu and Xia[27]. in petunia.

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

Treatments	Plant height (cm) at full bloom	Stem diameter (mm) at full bloom	Number of leaves plant^{-1} (60 DAP)	Total number of branches plant^{-1} at full bloom
T_1-100% RDF + *Piriformospora indica* inoculated to seeds	50.71	13.63	90.21	97.76
T_2-75% RDF + *Piriformospora indica* inoculated to seeds	58.29	14.47	105.20	97.09
T_3-50% RDF + *Piriformospora indica* inoculated to seeds	56.54	14.56	92.58	76.16
T_4-100% RDF + *Piriformospora indica* inoculated to seedling roots at the time of transplanting	50.24	13.37	89.55	99.07
T_5-75 % RDF + *Piriformospora indica* inoculated to seedling roots at the time of transplanting	49.83	14.11	84.61	99.43
T_6-50 % RDF + *Piriformospora indica* inoculated to seedling roots at the time of transplanting	52.21	13.21	82.89	75.04
T_7-75 % RDF + *Piriformospora indica* inoculated before transplanting	48.35	13.08	85.13	71.51
T_8- 75 % RDF + *Piriformospora indica* after pinching (40 days after transplanting)	45.21	12.60	65.02	71.04
T_9- Control	45.17	11.90	59.42	63.04
SEM ±	2.76	0.46	1.54	0.91
CD (*P*= 0.05)	8.31	1.41	4.65	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

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